

# ENTOMON

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ASSOCIATION FOR ADVANCEMENT OF ENTOMOLOGY

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## ENTOMON

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**Reprints:** The present policy is to supply 25 reprints *gratis*.

## **Report of the 20th General Body Meeting of the Association for Advancement of Entomology**

The 20th General Body Meeting of the Association was held on 14th August at 4.30 p.m. at the auditorium of Hotel Prasanth, Thiruvananthapuram with Dr. K. S. S. Nair, President of the Association in chair. 16 members were present. The members observed one minute silence to pay homage to the be-reaved soul of Prof. M. R. G. K. Nair, the former President of AAE. Then the Secretary--Treasurer presented the minutes of the 19th General Body Meeting which was unanimously approved and passed. This was followed by the presentation of the report of Association for the year 1998–1999 and the audited statement of accounts of the association for the financial year 1998–1999 and were passed unanimously.

*Decisions:* The following decisions were taken: Decided to enhance remuneration of the office assistant from Rs. 800 to Rs. 1000/- p.m. Decided to admit two more new life members. It was approved to send a proforma to all life members of the Association to furnish their biodata to prepare a new directory. Decided to open a new bank account at SBI/any nationalised bank. The major subject of discussion was about the conduct of an International Entomology Conference “Entomocongress-2000” to be held in November 2000. All members of the Executive Committee and selected representations from Universities/Research Institutions of South India were decided to be the Organizing Committee of the Conference. This organizing committee was approved in this meeting. It was decided to circulate the first announcement of the conference at the earliest. Members of the general body meeting entrusted the Organizing Committee to meet very soon to discuss the budget of the conference and how to raise funds from various organizations. Finally the members of general body decided that the existing executive committee will continue for the current year, since all members of the executive committee are ex-officio members of the newly constituted Organizing Committee.

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The Meeting came to a close by 6 p.m.



## Regulation of Protein and RNA Synthesis in Male Accessory Glands of *Spodoptera litura* (Fabr.) (Lepidoptera : Noctuidae) by Juvenile Hormone and Juvenoids

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**ABSTRACT:** A comparative study on the regulation of protein and RNA synthesis in male accessory reproductive gland (MARG) of the tobacco caterpillar, *Spodoptera litura* by juvenile hormone III and juvenoids was undertaken. Quantitative estimation of TCA precipitable proteins and electrophoretic profile indicated increase in the number and concentration of proteins as development progresses. Injection of 10 µg JH III into pupae increased synthesis of proteins that would normally be synthesised in fully developed gland of the adult insect. *In vivo* studies also established 11 day old pupae as the most responsive to JH III treatment. Short term *in vitro* organ culture technique, using tritiated precursors, was adopted to follow protein and RNA synthesis. JH III at  $10^{-5}$  to  $10^{-7}$  M was found to be physiologically active, stimulating both protein and RNA synthesis. Higher concentration ( $10^{-4}$  M) was inhibitory. Actinomycin D completely inhibited JH stimulation of RNA synthesis indicating JH stimulation of RNA thus indicating that JH stimulates *de novo* synthesis of proteins in *S. litura* MARG. The action of juvenoids, methoprene and fenoxycarb was not comparable to that of JH III at equimolar concentrations. At higher concentrations, both the compounds were inhibitory while lower concentrations had no positive effect on the synthesis of either proteins or RNA. © 2000 Association for Advancement of Entomology

**KEYWORDS:** Juvenile hormone, juvenoids, accessory glands, *Spodoptera litura*.

### INTRODUCTION

The secretions of male accessory reproductive gland (MARG) are important for the reproductive success of insects. Juvenile hormone (JH) regulates the synthetic and secretory activity of MARG in several insects *viz.*, *Drosophila melanogaster* (Yamamoto *et al.*, 1988), *Rhodnius prolixus* (Gold and Davey, 1989) and *Blatella germanica* (Piulachs *et al.*, 1992). *In vitro* studies have resulted in identification of a JH receptor in MARG of *Drosophila* (Shemshedini and Wilson, 1993). Extensive work

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on *Melanoplus sanguinipes* showed that while synthesis of some specific proteins in MARG secretions is influenced by JH (Cheesman and Gillott, 1988; Gillott and Gaines, 1992), some other proteins are regulated by 20-hydroxy ecdysone (Ismail and Gillott, 1995). Lepidopterans have not been subjected to such detailed studies. Influence of ecdysterone on MARG and stimulation of protein synthesis in *Chilo partellus*, *Opesina arenosella* and *Spodoptera litura* has been reported (Sridevi *et al.*, 1989; Geetha *et al.*, 1990 and Ismail *et al.*, 1993). Such reports on variation in hormonal regulation of synthetic activity of the gland necessitate detailed investigations using different insect species to elucidate the mechanism of regulation.

In the present investigation, therefore, the regulation of protein and RNA synthesis in MARG of the tobacco caterpillar, *Spodoptera litura* by JH III is studied. Further, the action of two juvenoids, methoprene and fenoxycarb is compared with that of JH III.

## MATERIALS AND METHODS

### Insects

A continuous colony of *S. litura* was maintained in the laboratory (25 ± 1 °C, 14 h light) on a semi-synthetic diet as previously described (Mane and Subrahmanyam, 1996). Development of MARG starts in late pupal stage (Sridevi *et al.*, 1989) therefore, synchrony in physiological development was ensured by separating out the pupae each morning at fixed time.

### Chemicals

JH III and  $\beta$ -ecdysone were obtained from Sigma, USA. Methoprene (Technical) and fenoxycarb (25% WP) were from Zoecon Corporation, USA and Ciba Geigy, Switzerland, respectively. For *in vitro* studies, molar stock solutions of JH III,  $\beta$ -ecdysone and methoprene were prepared in hexane. The active ingredient of fenoxycarb was extracted into acetone and redissolved in ethanol. All stock solutions were stored at -70 °C till use. The working solutions were prepared in 2 ml of the incubation medium 3-(N-morpholino) propane sulfonic acid buffer, pH 7.0 (MOPS) containing 0.1 mM CaCl<sub>2</sub>. <sup>3</sup>H-leucine (Specific activity 50,000 mCi/m mol) and <sup>3</sup>H-uridine (Specific activity 16,000 mCi/m mol) were obtained from Bhabha Atomic Research Center, Bombay.

### Estimation of protein

Ten micrograms of JH III in 10  $\mu$ l olive oil: ethanol (3 : 1) were injected per individual of 8, 10 and 12 days old pupae and freshly emerged adults. The glands were carefully dissected out 12, 24 and 48 h after each treatment and rinsed in saline. One pair of glands per replication and five replications were assayed. Control insects were injected 10  $\mu$ l of olive oil: ethanol (3 : 1) on day 8 of pupal stage and glands were collected subsequently as stated above. The glands were processed in trichloroacetic acid (TCA) as described by Sridevi and Ray (1988) to obtain precipitate of the proteins which was dissolved in 100  $\mu$ l of 0.1 N NaOH. An aliquot of this solution was used for protein estimation by the method of Lowry *et al.* (1951).

### Electrophoresis

JH III treatment and sample collection was the same as detailed above. Two gland pairs, comprising one replication were rinsed in distilled water and macerated in 100  $\mu$ l of 0.1 M phosphate buffer (pH 7.2) containing 0.05 M KCl, 5.0 mM EDTA and 1.0 mM PMSF. The suspension was centrifuged at 9,000 rpm for 20 min. The clear supernatant was collected and an aliquot of 25  $\mu$ l was mixed with 2x Laemmli's sample buffer, pH 6.8. MARG polypeptides were separated by electrophoresis on 4–18% linear gradient of polyacrylamide gel having SDS according to Laemmli (1970). Standard wide range molecular weight marker proteins (Sigma) were also run. The protein bands were visualised by silver staining procedure (Demerval *et al.*, 1987) and the relative mobilities of the protein bands with reference to the dye front were calculated to estimate the molecular weights.

### Protein synthesis *in vivo*

To examine the influence of JH III on protein synthesis in MARG under *in vivo* conditions, 11 day old pupae ( $24 \pm 2$  h prior to eclosion), freshly emerged and 8 h old adults were selected. The insects were injected with 10  $\mu$ g JH III. Controls received 10  $\mu$ l of carrier only. After a 10 h pre-incubation, each insect was injected with 3  $\mu$ Ci  $^3$ H-leucine followed by a further 2 h incubation. The glands were dissected and processed as described earlier. Two gland pairs constituted a replication. The entire 100  $\mu$ l of 0.1 N NaOH containing the dissolved precipitate was added to 5 ml scintillation fluid (Riatron). After a brief vortexing, the radio-label incorporated into protein was counted as disintegrations per minute (DPM) in a Tricarb liquid scintillation analyser (Packard 1600 TR) for 2 min at 48% efficiency.

### *In vitro* protein and RNA synthesis

Based on the protein content and *in vivo* effect of JH III, 11 day old pupae (24–30 h prior to eclosion) in the weight range of 275–300 mg were selected for all *in vitro* experiments. Two pairs of accessory glands constituting one replication were dissected in MOPS buffer and transferred into siliconised glass vials containing 60  $\mu$ l MOPS buffer (Shemshedini and Wilson, 1993), to which different concentrations of JH III/juvenoids were added and incubated at  $26 \pm 0.5$  °C for 30 min. For studies on protein synthesis, 1  $\mu$ Ci of  $^3$ H leucine was added while for RNA synthesis studies, 3  $\mu$ Ci of  $^3$ H uridine were added and incubated for further 90 min.

To measure protein synthesis, the glands were macerated in 50  $\mu$ l distilled water and processed as described earlier for protein estimation studies. The entire 100  $\mu$ l of 0.1 N NaOH containing the dissolved precipitate was added to 5 ml scintillation fluid and counted for 2 minutes in liquid scintillation analyser. To measure RNA synthesis, the glands were rinsed in distilled water, lysed in 50  $\mu$ l Laemmli's sample buffer (pH 6.8) of which 20  $\mu$ l was spotted on Whatman DE 81 adsorbing filter disc. The discs were washed as described by Maniatis *et al.* (1982) to eliminate the non-specific radioactivity. The dried discs were placed in glass counting vials containing 10 ml scintillation fluid and counted for 2 min.

### Effect of inhibitor, copulation and $\beta$ -ecdysone

To determine the relation between JH stimulated protein and RNA synthesis, the effect of inhibitor, actinomycin D was studied. The glands were dissected out and transferred to 60  $\mu$ l buffer containing JH III ( $10^{-5}$  M) and actinomycin D (2.5  $\mu$ g). After pre-incubation for 1 h, radioactivity was added and incubated for 90 min. *In vitro* protein synthesis was also compared between glands from freshly emerged (unmated) and mated males. Effect of  $\beta$ -ecdysone was studied by pre-incubating the glands in 60  $\mu$ l buffer containing  $10^{-6}$  or  $10^{-7}$  M of  $\beta$ -ecdysone.

## RESULTS

### Influence of JH III on protein concentration

The protein content of MARG increased from the 8th day of pupal stage to the adult stage (Table 1). On day 8 the protein content was 641.7  $\mu$ g gland $^{-1}$ . There was a significant increase in the subsequent 4 days and the concentration remained more or less at the same level with only a slight increase in the freshly eclosed adults. Injection of JH III was found to increase the protein concentration when compared with the respective controls at all stages of development. Significant increase in concentration was noted especially when 10 day old pupae or freshly eclosed adults were used. Treatment of 10 day old pupae resulted in 26.0 per cent increase in protein concentration over control within 24 h of treatment and after 48 h the increase was 74.8 per cent. Similarly, treatment soon after eclosion increased the protein content by 18.0 and 77.5 per cent over control at 12 and 48 h, respectively.

### Electrophoretic pattern of proteins

The protein profile of MARG of normally developing *S. litura* presents a gradual increase in the number of bands as the development progresses (Plate 1a, Table 2a). Proteins are categorized as high (150–80 kD), medium (79–35 kD) and low (<35 kD) molecular weight proteins. In day 8 pupae, the protein content was low as can be seen from the appearance of only 8 low intensity bands. The number of protein bands increased later and in the adult stage when the gland development was complete and synthetically active, as many as 23 protein bands can distinctly be seen, of which 8 are new proteins appearing only after adult eclosion.

Treatment with JH III caused appreciable change in the banding pattern of proteins (Plate 1b, Table 2b). Six additional high molecular weight proteins were synthesised following injection of JH III into two early stages of pupae whereas, 6 high, 2 medium and 5 low molecular weight proteins were additionally formed when 12 day old pupae were injected with 10  $\mu$ g JH III. Injection of JH III into adults had no further influence on protein synthesis.

TABLE 1. Effect of JH III on total protein concentration in MARG of *S. litura*

Stage	Control	12 h	JH III treatment 24 h	48 h
			Protein content ( $\mu\text{g}/\text{insect}$ )	
8 day Pupae	641 $\pm$ 14.3 <sup>ac</sup>	650 $\pm$ 25.0 <sup>d</sup>	725.0 $\pm$ 52.0 <sup>d</sup>	758 $\pm$ 14.4 <sup>a</sup>
10 day Pupae	758 $\pm$ 14.4 <sup>c</sup>	958.3 $\pm$ 14.4	1116.7 $\pm$ 15.0 <sup>e</sup>	1325.0 $\pm$ 66.1 <sup>f</sup>
12 day Pupae	1100.0 $\pm$ 24.0 <sup>bd</sup>	1133.3 $\pm$ 12.4 <sup>b</sup>	1175.0 $\pm$ 43.3 <sup>be</sup>	1216.0 $\pm$ 14.3 <sup>bf</sup>
0 day Adult	1108.3 $\pm$ 15.6 <sup>d</sup>	1308.3 $\pm$ 80.3	1666.7 $\pm$ 32.4	1966.7 $\pm$ 12.6
Each value is the mean ( $\pm$ SE) of 3 replications				
	Test	Treatments	Interactions	
	FCRD	Significant	Significant	
	CD	141.35	141.35	
Values having the same letter are not statistically significant				

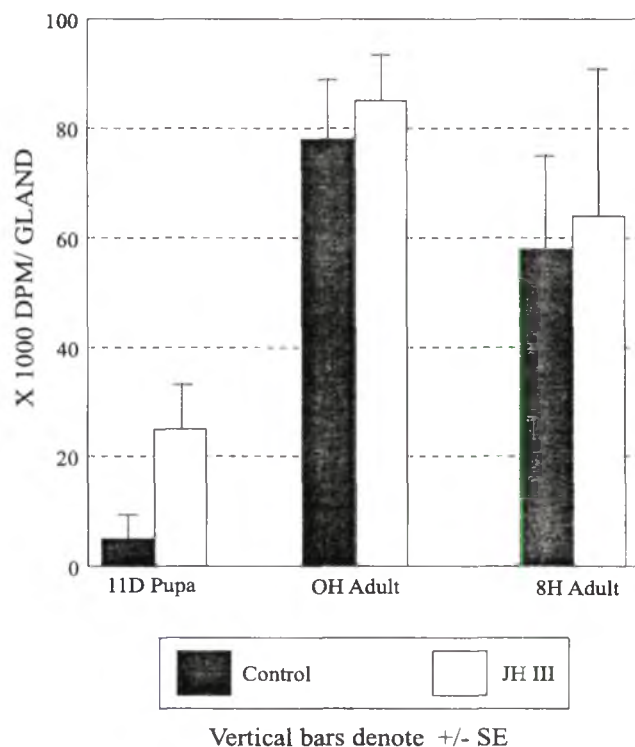


FIGURE 1. Effect of JH III on *in vivo* protein synthesis in MARG.

#### Effect of JH III on *in vivo* protein synthesis

Protein synthesis was relatively low in 11 day old pupae (4585 DPM/gland) but increased extremely rapidly in the next 48 h at the time of adult eclosion (77585 DPM/gland), as evident from Fig. 1. Synthesis remained high 8 h after eclosion. However, it had declined compared to the earlier stage. Injection of 10 mg JH III increased protein synthesis in 11 day old pupae by 5.2 times over control (24008 DPM/gland), whereas it had no appreciable influence on the adults. The study thus revealed that JH III has most pronounced stimulatory effect on MARG proteins of 11 day old pupae. The *in vitro* studies were hence conducted at this stage.

#### Effect of JH III on the *in vitro* protein and RNA synthesis

##### Protein synthesis

Addition of JH III in the range of  $10^{-4}$  to  $10^{-9}$  M into the incubation medium led to increased protein synthesis in MARG (Table 3). At the lower concentrations the synthesis was relatively stable and it was about 1.3 times higher than in control. It got significantly elevated by about 3.0 and 5.0 times at  $10^{-6}$  and  $10^{-5}$  M, respectively. At

TABLE 2A. Protein profile of *S. litura* MARG at different developmental stages

Molecular Weight (kD)				
8 Day Pupa	10 Day Pupa	12 Day Pupa	0 Day Adult	2 Day Adult
—	—	—	—	151.3*
—	141.2	—	141.2	141.2
123.0	123.0	125.9	125.9	125.9
—	109.6	109.6	109.6	109.6
104.7	104.7	104.7	104.7	104.7
—	—	—	100.0	97.7*
—	89.1	87.0	87.0	87.0
—	—	—	83.1	81.3*
74.1	74.1	74.1	74.1	74.1
—	70.8	69.2	69.2	69.2
—	—	66.0	64.6	64.6
—	—	61.6	58.9	58.9
—	—	54.9	54.9	54.9
51.3	51.3	51.3	51.3	51.3
—	—	—	49.0	49.0*
42.7	42.7	—	42.7	42.7
—	—	40.7	40.7	—
—	—	38.0	38.0	38.9
—	—	—	—	36.9*
34.6	34.6	34.6	33.9	33.9
32.3	32.3	30.9	30.9	30.0
—	—	—	28.2	28.2*
—	—	—	25.7	24.0*
22.9	22.9	22.3	22.4	22.4
—	—	20.4	—	—

\* Protein bands observed only in adult MARG.

$10^{-4}$  M, the protein synthesis decreased though it was still 1.4 times higher than in control. Effect of the two juvenoids, methoprene and fenoxycarb on protein synthesis was different from that of JH III when tested at comparable concentrations (Table 3). Both the compounds at the highest concentration tested ( $10^{-5}$  M) were found to be strongly inhibitory, effect of fenoxycarb being more pronounced. At the lower concentrations there was only a marginal increase in protein synthesis compared to control.

On comparing the response of the glands to juvenoids with that of JH III it is seen that protein synthesis was 5.7 and 30 times less under the influence of methoprene and fenoxycarb, respectively. Lower concentrations of methoprene resulted in slightly higher synthesis compared to JH III whereas, lower concentrations of fenoxycarb ( $10^{-6}$  to  $10^{-8}$  M) were also inhibitory. Synthesis was 3.0 to 1.2 times lower than in glands exposed to JH III.

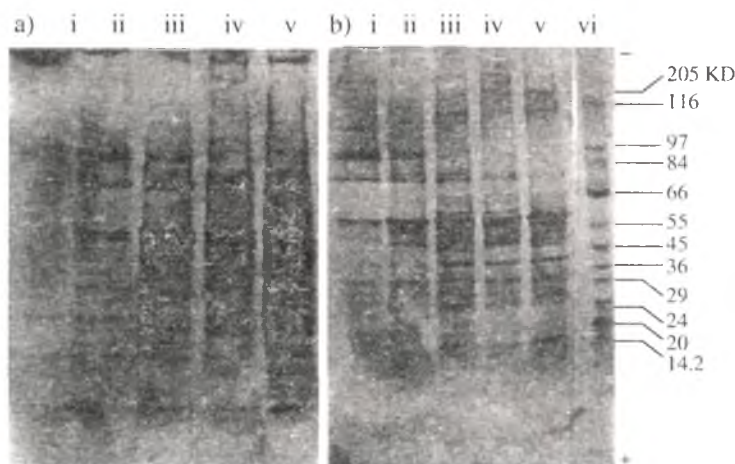
TABLE 2B. Effect of JH III on protein profile of *S. litura* MARG

Molecular Weight (kD)				
8 Day Pupa	10 Day Pupa	12 Day Pupa	0 Day Adult	2 Day Adult
—	218.8	218.8	218.8	—
213.8	—	—	213.8	204.2
182.0	—	195.0	195.0	190.5
178.0	178.0	—	182.0	—
166.0	159.5	169.8	169.8	169.8
151.3	151.3	—	—	—
—	141.2	141.2	141.2	141.2
—	—	—	131.8	—
125.9	125.9	125.9	125.9	125.9
—	120.2	120.2	—	120.2
—	—	112.2	117.5	117.5
—	—	112.2	117.5	117.5
—	—	100.0	—	109.6
97.7	97.7	97.7	97.7	97.7
—	87.1	87.1	—	—
81.3	81.3	81.3	81.3	81.3
—	77.6	—	—	77.6
—	66.1	66.1	66.1	66.1
—	63.1	61.6	63.1	63.1
—	—	57.5	57.5	57.5
52.4	52.4	52.4	52.4	52.4
—	48.0	49.0	49.0	49.0
45.7	—	45.7	45.7	45.7
—	43.6	42.7	43.6	43.6
40.7	—	40.7	—	40.7
—	35.0	36.3	36.3	36.3
30.2	30.2	30.9	30.9	30.9
27.0	27.0	28.2	27.0	27.0
25.7	25.1	25.1	24.0	25.1
19.1	19.1	20.0	20.4	20.4
—	—	18.1	19.0	19.0
17.8	17.8	17.8	17.0	17.8
—	16.6	16.6	—	—
—	—	15.8	15.8	15.8

*RNA synthesis*

RNA synthesis under the influence of JH III also showed an increasing trend with increase in concentration from  $10^{-9}$  to  $10^{-5}$  M (Table 4). The increase in synthesis was significant at  $10^{-7}$  to  $10^{-5}$  M, being 2.8 to 3.8 times higher than in control. In contrast, the two juvenoids had an inhibitory effect. Methoprene at the two higher concentrations resulted in 1.6 and 4.2 times lower RNA synthesis. Fenoxycarb at the the highest concentration tested ( $10^{-5}$  M) caused 4.4 times lower synthesis than in

PLATE 1



Electrophoretic profile of male accessory reproductive gland (MARG) (proteins) (Silver stained SDS-PAGE (4–18% linear gradient gels). (a) Control *S. litura*: Lane i – 8 day pupa; Lane ii – 10 day pupa; Lane iii – 12 day pupa; Lane iv – 0 day adult; Lane v – 2 day adult. (b) JH III treated *S. litura*: Lane i – 8 day pupa; Lane ii – 10 day pupa; Lane iii – 12 day pupa; Lane iv – 0 day adult; Lane v – 2 day adult; Lane vi – Standard molecular weight marker proteins (205–14.2 kD).

control. The effect when compared with that of JH III suggests that RNA synthesis under the influence of methoprene was 6.3 and 1.3 times lower. The difference was more pronounced in case of fenoxycarb where synthesis was 16.8 to 1.3 times lower than that in JH III treated glands at identical concentrations.

#### *Effect of inhibitors, copulation and $\beta$ -ecdysone*

To determine if JH induced protein synthesis is dependent on *de novo* RNA synthesis, the effect of actinomycin D on protein and RNA synthesis was studied. As seen from Table 5, actinomycin D completely inhibited JH stimulation of RNA synthesis while protein synthesis under such condition was 1.6 times lower than in control. Protein synthesis in MARG of both copulated and uncopulated males was found to be higher than in 11 day old pupae but there was no significant difference in the level of synthesis between copulated and uncopulated males. On exposure of MARG to  $\beta$ -ecdysone, a significant increase in protein synthesis was noticed at  $10^{-6}$  M. However, it did not stimulate the RNA synthesis.

#### DISCUSSION

The increasing trend in protein content of MARG indicates the progressive development of competence for secretion. A steady increase in tissue weight and protein content of *S. litura* MARG, beginning from the 8th day of the pupal stage, with a maximum in 10 h old adult, was reported by Sridevi and Ray (1988). Stimulation of MARG

TABLE 3. Effect of JH III and juvenoid treatments on *in vitro* protein synthesis in MARG of *S. litura*

Treatment	Concentration (M)	Protein synthesis	
		DPM/gland	DPM/mg protein
JH III	10 <sup>-9</sup>	27627 ± 6367	36447 ± 8399
	10 <sup>-8</sup>	28938 ± 9482	38177 ± 12509
	10 <sup>-7</sup>	28582 ± 11049	37707 ± 14577
	10 <sup>-6</sup>	63293 ± 14723*	83501 ± 19424*
	10 <sup>-5</sup>	110671 ± 31968*	146004 ± 42174*
	10 <sup>-4</sup>	30081 ± 3780	39684 ± 4987
Methoprene	10 <sup>-9</sup>	43870 ± 12663	57876 ± 16705
	10 <sup>-7</sup>	35262 ± 11250	46519 ± 14973
	10 <sup>-5</sup>	19537 ± 8185	25774 ± 10798
Fenoxycarb	10 <sup>-8</sup>	25006 ± 13570	32989 ± 17902
	10 <sup>-7</sup>	31411 ± 10863	41439 ± 14332
	10 <sup>-6</sup>	20653 ± 8522	20785 ± 13979
	10 <sup>-5</sup>	3667 ± 1579**	4838 ± 2083**
Control	—	21200 ± 3675	27968 ± 4848

Protein synthesis is represented in DPM of <sup>3</sup>H-Leucine incorporated into protein in 90 min. The values represent mean ± SE of minimum 5 replications.

\* Significantly different from control ( $P = 0.05$ )

\*\* Significantly differently from control ( $P = 0.01$ )

protein synthesis by JH III, as observed in this study, was reported in other insects (Cheesman and Gillott, 1988). Ismail and Gillott (1995) reported a gradual acquisition of competence to respond to JH in the MARG of *Melanoplus sanguinipes*.

Injection of JH III into pupae of *S. litura* accelerated the gland development and rapidly increased the number of protein bands. Even some of the proteins that would normally appear in adult stage appeared in pupal stage. This response to JH III is remarkable since the pupae have low level of endogenous JH due to inactive corpus allatum (CA). However, treatment of adults had no additional effect probably due to the reactivation of CA after eclosion and consequent raise in endogenous JH level. Thus, MARG of 10–11 day old pupae responded maximally to JH III. *In vivo* studies by Sridevi *et al.* (1989) suggest that JH I has no effect on protein synthesis in *S. litura* MARG. Gold and Davey (1989) found that JH III was ten times more effective than JH I in restoring protein synthesis in MARG of alletectomised adults of *Rhodnius prolixus*, lending support to this study.

*In vitro* study on protein and RNA synthesis indicated that JH III in the range of 10<sup>-7</sup> to 10<sup>-5</sup> M is physiologically active while higher concentrations may be inhibitory. Such a trend in protein synthesis under the influence of JH III was also reported by Yamamoto *et al.* (1988) in case of *D. melanogaster*, wherein increase over control at 10<sup>-9</sup> M was 300 per cent. The two juvenoids, methoprene and fenoxycarb inhibited

TABLE 4. Effect of JH III and juvenoids in *in vitro* RNA synthesis in MARG of *S. litura*

Treatment	Concentration (M)	RNA synthesis (DPM/gland)
JH III	$10^{-9}$	$8805 \pm 5343$
	$10^{-8}$	$10186 \pm 4001$
	$10^{-7}$	$16872 \pm 3303^*$
	$10^{-6}$	$17103 \pm 5181^*$
	$10^{-5}$	$22943 \pm 6459^*$
Methoprene	$10^{-9}$	$6474 \pm 2030$
	$10^{-7}$	$1405 \pm 786^*$
	$10^{-5}$	$3631 \pm 819$
Fenoxycarb	$10^{-8}$	$7563 \pm 2397$
	$10^{-7}$	$11623 \pm 1621$
	$10^{-6}$	$7622 \pm 5058$
	$10^{-5}$	$1360 \pm 790^*$
Control	—	$5955 \pm 1182$

RNA synthesis is represented in DPM of  $^3\text{H}$ -uridine incorporated into RNA in 90 min.

The values represent mean  $\pm$  SE of minimum 5 replications

\*Values are significantly different from control ( $P = 0.05$ )

TABLE 5. Effect of copulation,  $\beta$ -ecdysone and inhibitors on protein and RNA synthesis in MARG of *S. litura*

## (a) Protein synthesis

Treatment	Protein synthesis	
	DPM/gland	DPM/mg protein
Uncopulated male	$110226 \pm 9039^{**}$	$145417 \pm 11925^{**}$
Copulated male	$90009 \pm 17375^{**}$	$126785 \pm 22018^{**}$
$\beta$ -ecdysone $10^{-6}$ M	$76941 \pm 15791^*$	$101505 \pm 20833^*$
$\beta$ -ecdysone $10^{-7}$ M	$38620 \pm 5756$	$50950 \pm 7593$
JH III + Actinomycin D ( $10^{-5}$ M) (2.5 $\mu\text{g}$ )	$13332 \pm 5444$	$17587 \pm 7182$
Control	$21200 \pm 3675$	$27968 \pm 4848$

## (b) RNA synthesis

Treatment	RNA synthesis DPM/gland
$\beta$ -ecdysone $10^{-7}$ M	$5498 \pm 3507$
JH $10^{-5}$ M + Actinomycin D (2.5 $\mu\text{g}$ )	$248 \pm 187^{**}$
Control	$5955 \pm 1182$

protein as well as RNA synthesis. Such an inhibitory effect, though not reported earlier, could be partly explained based on the binding efficiency of juvenoids to JH receptors. The binding efficiency of methoprene to JH receptor in MARG of *D. melanogaster* is 100 times less than that of JH III (Shemshedini *et al.*, 1990). In *M. sexta*, methoprene and JH III bind to two different sites in the epidermal cell nuclei (Osir and Riddiford, 1988). Hence the physiological effects of JH can not be totally mimicked by juvenoids. Shemshedini and Wilson (1993) reported the presence of two binding proteins having specific affinity to JH III, one in the cytosol and one in the nucleus. They also demonstrated the elevated synthesis of RNA by JH III in MARG, similar to the present study. Hence, JH may operate through the nucleus to effect at least part of its action.

The inhibitory effect of actinomycin D on JH induced RNA synthesis suggests that JH stimulates *de novo* synthesis of proteins. Copulation did not influence protein synthesis in *S. litura* MARG. This aspect has so far not been studied. In *D. melanogaster* it is established that copulation stimulates RNA and protein synthesis and the effect is comparable to that of  $10^{-9}$  M JH III *in vitro* Schmidt *et al.*, 1985 and Yamamoto *et al.*, 1988. Males of *S. litura* mate soon after emergence. The reactivation of CA may synchronize with adult emergence to influence MARG. Further, it is well known that in lepidopterans, JH III is synthesized from its precursors by MARG in the adult stage. Hence, copulation has no additional influence on protein synthesis. This study also demonstrates that  $\beta$ -ecdysone does not stimulate RNA synthesis though it could stimulate protein synthesis. In *D. melanogaster* combined treatment with  $\beta$ -ecdysone and JH III stimulated protein synthesis not more than JH III alone (Yamamoto *et al.*, 1988) and it may influence the synthesis of only certain polypeptides as in *M. sanguinipes* (Ismail and Gillott, 1995).

The study thus brings out that the two juvenoids, methoprene and fenoxycarb do not entirely mimic the action of JH III on the MARG of *S. litura*. It also suggests that JH III acts both on cytoplasm and nucleus. Identification of JH receptor proteins would be of paramount importance in further understanding the action of JH at the molecular level.

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## Developmental Changes in Male and Female Reproductive Organs of the Pyrgomorphid Grasshopper, *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae) with Age of the Adults

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**ABSTRACT:** Body weights of unmated female *Z. variegatus* are generally higher than those males, particularly from week 4 to 15. At every age, females were longer than those of males. From 5 weeks of age, the mean number of mature eggs and egg weight significantly increased with age of female. Spermathecal weight similarly increased with age, indicating continuous maturation of the gland.

The mean weight of testes, and accessory gland-seminal vesicle bundles significantly increased with age of adult male demonstrating a continuous development in an unmated maturing adult *Z. variegatus*. © 2000 Association for Advancement of Entomology

**KEYWORDS:** Development, reproductive organs *Zonocerus variegatus*.

### INTRODUCTION

Insemination in the acridid grasshoppers involves the transfer of spermatozoa and seminal fluid from the seminal vesicles and accessory glands of matured adult male into the spermatheca of sexually mature females. Spermatophore which transfers the sperm (Engelmann, 1970) has its origin from the secretions of the accessory glands of male *Melanoplus sanguinipes* (Pickford and Gillot, 1972). Insects are made of cells which are not distinguishable from those of higher animals. They grow old, manifest aging processes and die afterwards. However, little attention has been paid to changes occurring, subsequent to the imaginal moult, especially to specific tissues in insects (Sohal, 1985). It is against this background that the present study investigates the developmental changes in the testes and accessory gland-seminal vesicle bundles of the male and in the ovaries and spermatheca of female *Zonocerus variegatus* L., a pest of cassava in West and Central Africa (Page, 1978) at different age of the adult.

### MATERIALS AND METHODS

Nymphs at various instars of the dry season population of *Z. variegatus* were collected with sweep net from different locations of Obafemi Awolowo University campus, Ile-Ife, Nigeria. They were maintained in a cage (38 × 52 × 82 cm) in the insectary at

$28 \pm 2^\circ\text{C}$  and 70–75% RH. They were provided with *Chromolaena odorata* (Siam Weed) and *Manihot esculenta* (cassava) shoot which served as food and as perches for moulting purposes. The newly emerged adult males and females were removed from the cage containing the nymphs. They were marked with Magic Ink marker on the pronotum and sometimes on the forewings, to determine their ages and were put in separate cages ( $36 \times 37 \times 50$  cm) according to sex. The adults were used for experiments at various stages of development.

At weekly intervals, from the newly emerged adult, to one week old and up to 15 week-old adults, the lengths and body weights of five males and five females were taken at each age. The same set of males were dissected under an illuminated dissecting microscope to isolate the paired testes and the closely-adhered paired accessory glands-seminal vesicles bundle and their weights were recorded on Mattler balance. Females were similarly dissected to isolate the paired ovaries and spermatheca, and their weights were similarly recorded. The paired egg batches were removed and put in 10% ethanol for 48 h to facilitate the removal of tissues. The eggs were counted and their weights recorded.

## RESULTS AND DISCUSSION

### Body weight and length

The mean body weight of males fluctuated from emergence up to 15 week-old males at weekly intervals. In both males and females, the mean body weight increased significantly from emergence to week 15 insects respectively ( $F = 2.16$ ,  $P = 0.018$ ;  $F = 13.05$ ,  $P = 0.001$ ,  $df = 15, 64$ ). (Fig. 1). In females, there was noticeable increase in body weight from 6 week-old to week 15 insects. Body weights of females were generally higher than those of males probably due to egg maturation in the unmated female. There was no correlation between age and mean body length of male and female respectively ( $r = -0.114$ ,  $P = 0.156$ ;  $r = 0.054$ ,  $P = 0.348$ ) but the mean body weight of males and females increased significantly with age of insect ( $F = 7.77$ ,  $P = 0.001$ ;  $F = 9.86$ ,  $P = 0.0001$ ) respectively. At every age, the mean body length of female was significantly higher than those males ( $t = -10.17$ ,  $df = 79$ ,  $P < 0.0001$ ). (Fig.1). Body length was positively correlated with the body weight of male and female respectively ( $r = 0.543$ ,  $P = 0.0001$ ;  $n = 80$ ,  $r = 0.657$ ,  $P = 0.0001$ ,  $n = 55$ )

### Female reproductive organs

The mean body weight of female and positively correlated with the mean ovary weight, egg weight and egg number ( $r = 0.604$ ,  $P = 0.0001$ ;  $r = 0.388$ ,  $P = 0.002$ ,  $r = 0.583$ ,  $P = 0.0001$ ) respectively, indicating the contribution of the various organs in the determination of insect body weight for an unmated female at all ages. Egg number was positively correlated with ovary weight and egg weight respectively ( $r = 0.796$ ,  $P = 0.0001$ ;  $r = 0.749$ ,  $r = 0.0001$ ) suggesting a high mutual dependence on each other. There was general increase in egg number and it seems

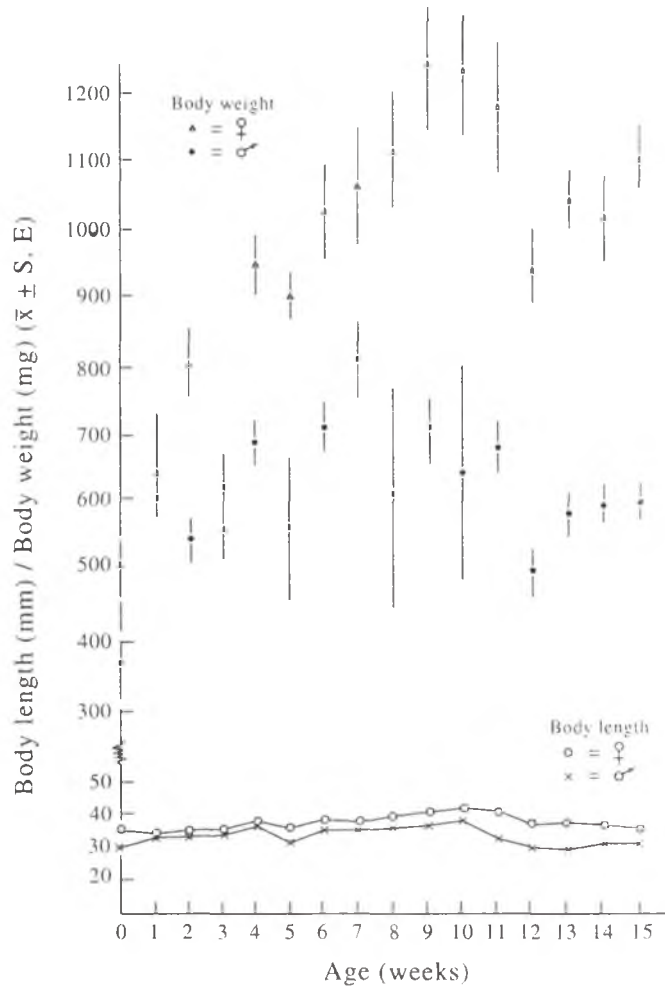


FIGURE 1. Relationship between age and mean body length and weight of adult male and female *Z. variegatus*. \*Standard error bars are small and not shown.

they are at different levels of development resulting in different weights of the eggs with age of insect. Ovary weight was positively correlated with the body length and egg weight ( $4 = 0.415$ ,  $P = 0.0001$ ,  $r = 0.808$ ,  $P = 0.0001$ ). There was significant increase in mane number of eggs and egg weight with age of adult from 5 weeks-old to week 15 insects (Table 1) The increase in number and weight of eggs was independent of female insemination, which is responsible for increase in egg number and maturation in several species of insects (Engelmann, 1970).

Accessory gland and seminal vesicle substances enhance ovarian maturation and egg laying (Pickford and Gillot, 1972; Leahy, 1973) but their absence in female

TABLE 1. Mean weights of ovaries, egg batches, spermatheca and egg number with age of *Z. variegatus*

Age (week)	Ovaries (mg $\pm$ S.E)	Egg batches (mg $\pm$ S.E)	Spermatheca (mg $\pm$ S.E)	Egg Number
0	23.68 $\pm$ 0.61	—	1.16 $\pm$ 0.04	—
1	40.24 $\pm$ 10.11	—	1.34 $\pm$ 0.06	—
2	40.78 $\pm$ 2.83	—	1.26 $\pm$ 0.27	—
3	31.60 $\pm$ 7.68	—	1.20 $\pm$ 0.23	—
4	52.90 $\pm$ 4.47	—	1.38 $\pm$ 0.07	—
5	172.24 $\pm$ 15.38	183.28 $\pm$ 30.91	1.72 $\pm$ 0.22	17.40 $\pm$ 2.66
6	319.40 $\pm$ 50.41	272.80 $\pm$ 41.26	1.34 $\pm$ 0.11	25.20 $\pm$ 3.93
7	341.16 $\pm$ 41.46	332.00 $\pm$ 42.43	1.28 $\pm$ 0.10	32.00 $\pm$ 3.54
8	370.80 $\pm$ 36.36	268.04 $\pm$ 40.82	1.28 $\pm$ 0.11	35.20 $\pm$ 5.40
9	565.26 $\pm$ 66.59	376.02 $\pm$ 19.67	1.30 $\pm$ 0.14	32.20 $\pm$ 2.46
10	480.12 $\pm$ 102.76	355.56 $\pm$ 61.13	1.30 $\pm$ 0.20	31.20 $\pm$ 5.08
11	478.88 $\pm$ 59.33	416.76 $\pm$ 46.59	1.10 $\pm$ 0.13	31.00 $\pm$ 3.36
12	576.56 $\pm$ 57.01	369.92 $\pm$ 44.34	1.60 $\pm$ 0.26	31.00 $\pm$ 4.15
13	526.86 $\pm$ 53.19	492.92 $\pm$ 44.34	2.00 $\pm$ 0.11	35.40 $\pm$ 5.24
14	401.52 $\pm$ 71.08	401.84 $\pm$ 57.62	1.72 $\pm$ 0.21	28.80 $\pm$ 4.24
15	564.26 $\pm$ 14.01	535.44 $\pm$ 27.81	1.76 $\pm$ 0.17	39.60 $\pm$ 2.94

did not prevent egg development in *Z. variegatus*. The mean ovary weight increased gradually from emergence up to 4 weeks-old insects and significantly from week 5 to 15 week-old insects ( $F = 20.0228$ ,  $P = 0.0001$ ;  $df = 15, 64$ ). The remarkable increase from week 5 insects is due to egg maturation in ovary and also coincides with commencement of mating (Youdeowei, 1974). The mean weight of an egg increases linearly with age of adult (Fig. 2). Adult *Z. variegatus* commence egg deposition from week 9 in the laboratory (personal communication). The females probably ensure the fertilization of mature eggs since the period correspond to a phase of continuous egg growth as shown in Fig. 2. The mean spermathecal weight increased significantly with increase in age of adults ( $F = 2.380$ ,  $df = 15, 64$ ,  $P = 0.009$ ). The spermathecal produces its own secretion in acridid grasshoppers (Gillot, 1991) and it seems to be accumulated in a developing unmated *Z. variegatus*.

#### Male reproductive organ

There was a variable but significant increase in the mean weight of testes as the adult insects mature ( $F = 7.88$ ,  $df = 15, 64$ ,  $P = 0.0001$ ). The paired testes seem to accumulate materials for transfer to the accessory gland seminal vesicle bundles. This is also reinforced by the existence of positive correlation between insect age and testes weight ( $r = 0.640$ ,  $P = 0.0001$ ) (Table 2). The mean weight of accessory gland seminal vesicle bundles was significant with age of insect ( $F = 7.07$ ,  $df = 15, 64$ ,  $P = 0.0001$ ) and was positively correlated with testes weight ( $r = 0.49$ ,  $P = 0.001$ ) and age of adult ( $r = .68$ ,  $P = 0.0001$ ). Muse (1993) demonstrated an accumulation of proteins in the accessory glands of unmated *Z. variegatus*. Increase in the seize of

TABLE 2. Mean weights of testes and accessory gland-seminal residue (ARG-SV) bundle with age of *Z. Variegatus*

Age (week)	Testes (mg $\pm$ S.E.)	ARG-SV (mg $\pm$ S.E.)
0	17.84 $\pm$ 4.40	8.32 $\pm$ 1.40
1	24.72 $\pm$ 3.29	10.22 $\pm$ 1.30
2	29.54 $\pm$ 5.32	10.36 $\pm$ 0.86
3	18.80 $\pm$ 0.81	11.94 $\pm$ 0.61
4	22.74 $\pm$ 2.56	11.96 $\pm$ 0.91
5	29.20 $\pm$ 1.31	11.76 $\pm$ 1.53
6	24.00 $\pm$ 2.70	12.94 $\pm$ 0.69
7	26.06 $\pm$ 2.51	15.92 $\pm$ 0.93
8	34.64 $\pm$ 2.88	16.80 $\pm$ 1.95
9	30.36 $\pm$ 2.66	18.42 $\pm$ 0.38
10	26.78 $\pm$ 1.36	17.24 $\pm$ 1.46
11	52.56 $\pm$ 4.34	25.20 $\pm$ 3.81
12	40.26 $\pm$ 2.98	13.82 $\pm$ 3.73
13	41.22 $\pm$ 5.51	18.82 $\pm$ 0.96
14	34.98 $\pm$ 4.94	20.12 $\pm$ 2.29
15	48.96 $\pm$ 5.13	22.80 $\pm$ 1.58

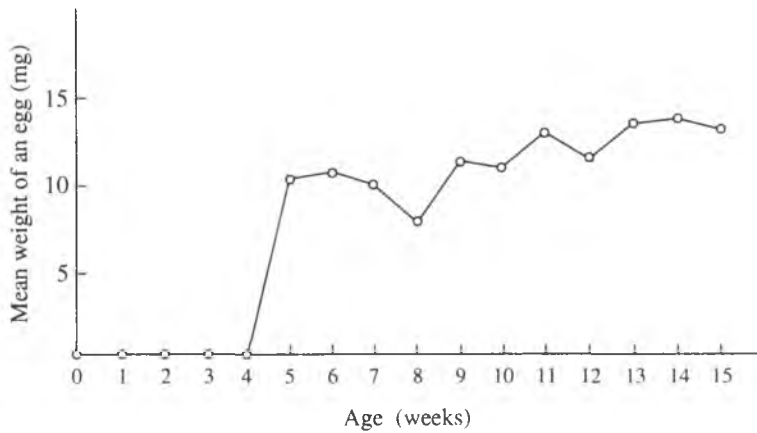


FIGURE 2. Changes in weight of an egg with age of adult female *Z. variegatus*.

accessory gland during male maturation has been described in *Schistocerca gregaria* (Dhadialla *et al.*, 1986). The present result demonstrates a continuous accumulation of substances in the accessory glands and seminal vesicles as the unmated insects increased in age. There was no correlation between the body weight and testes weight ( $r = 0.05$ ,  $P = 0.343$ ) but a weak one exists between the body weight and accessory gland seminal vesicle bundles ( $r = 0.28$ ,  $P = 0.006$ ). This further indicates the

accumulation function of the accessory gland seminal vesicle bundles in contrast with the paired testes.

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## Insecticidal Properties of Essential Oil of *Cannabis sativa* Linn. Against Mosquito Larvae

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**ABSTRACT:** Laboratory studies carried out with the essential oil of an indigenous plant, *Cannabis sativa* to evaluate its mosquito larvicidal properties revealed that the oil could induce 100.0 percent mortality at concentrations of 0.06, 0.1, 0.12 and 0.2 ml/litre of water in the larvae of *Culex tritaeniorhynchus*, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* respectively. The LC<sub>50</sub> & LC<sub>90</sub> values estimated for *Cx. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were 0.0101 & 0.0295, 0.026 & 0.0749, 0.0273 & 0.0919 and 0.0453 & 0.1803 respectively. The essential oil of *C. sativa* plant was found to be more toxic to *Cx. tritaeniorhynchus* followed by *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The aqueous oil extract was found to be more toxic than the ethanolic extract. © 2000 Association for Advancement of Entomology

**KEYWORDS:** *Cannabis sativa*, oil, larvicide.

### INTRODUCTION

Wide spread development of insecticide resistance in mosquitoes and other arthropods of medical importance is a major problem faced in the control of vector-borne diseases. Besides, increasing public awareness about the contamination of environment by toxic chemical residues both in developed and developing countries and public perception about the use of eco-friendly methods for the control of pests of agricultural and public health importance has necessitated the search and development of non-chemical methods. Hartzell and Wilcoxon, 1941, undertook detailed survey of plant products to determine their insecticidal properties. Since then insecticidal properties of many plants came into limelight for the control of arthropods of medical importance.

*Cannabis sativa* Linn. (Family–Cannabinaceae) is an annual herb, which grows widely in most temperate and tropical areas of the world. The medicinal properties of *Cannabis* came into limelight during the reign of Chinese Emperor, Shen Nung

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TABLE 1. Results of laboratory evaluation of essential oil of *Cannabis sativa* against larvae of different species of mosquitoes

Mosquito species	LC <sub>50</sub> values	LC <sub>90</sub> values	Heterogeneity $\chi^2$ (df)*
<i>Cx. tritaeniorhynchus</i>	0.0101 (0.0089–0.0114)	0.0295	4.2897(3)
<i>Ae. aegypti</i>	0.026 (0.0231–0.0288)	0.0749	14.9483(5)
<i>An. stephensi</i>	0.0273 (0.0244–0.273)	0.0919	36.7507(5)
<i>Cx. quinquefasciatus</i>	0.0453 (0.0405–0.0502)	0.1803	23.4733(9)

df\* - Degree of freedom

about 300 BC, when its use was recommended for the treatment of a variety of diseases including rheumatism, malaria and constipation etc. (Dahiya, 1976). It has also been reported to be a rich source of fiber (hemp) and psychoactive substance (World Health Organization, 1971). In India, *Cannabis* has been used in indigenous systems of medicine for many centuries (Chopra and Chopra, 1957). Jalees *et al.*, (1993) reported the mosquito larvicidal properties of ethanolic extract of essential oil of *C. sativa*.

Present communication deals with the laboratory studies carried out with the aqueous oil extract of *C. sativa* against the larvae of *Cx. tritaeniorhynchus*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes

#### MATERIALS AND METHODS

*C. sativa* plants were collected from in and around the National Zoological Park, Delhi. Freshly harvested flowering plants were chopped off finely and subjected to hydro-distillation in Clavenger's apparatus to obtain the essential oil.

The laboratory bio-assay of oil was undertaken against the larvae of laboratory strain of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* mosquitoes using WHO techniques. The tests were conducted at ambient room temperature,  $27 \pm 1^\circ\text{C}$  and relative humidity 75–85 percent. A series of concentrations of essential oil ranging from 0.005 to 0.2 ml/litre of water were made by dissolving it in 0.5 ml of acetone. For each concentration four replicates were run separately for each species of mosquitoes and control tests were run parallelly with 0.5 ml acetone in 250 ml of water. Twenty five late 3rd or early 4th instar larvae were exposed in 500 ml capacity beaker containing required concentration of the essential oil in 250 ml of water. Brewer's yeast was provided as larval food. Mortality counts were made after 24 hours of exposure period. The tests showing more than 20 percent mortality in control experiments were discarded and repeated. In case control mortality ranged

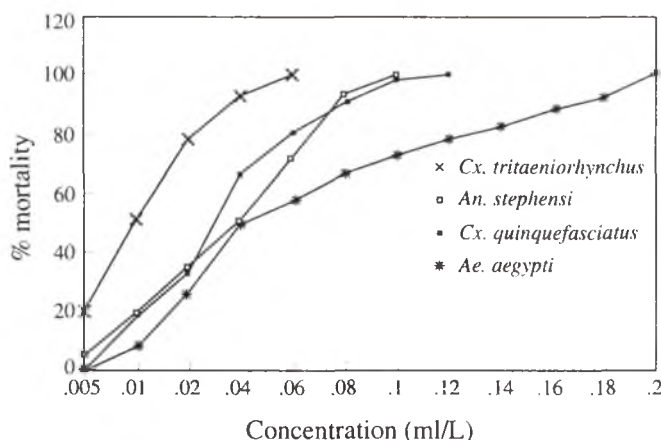


FIGURE 1. Laboratory evaluation of oil of *Cannabis sativa* plant against larvae of four species of mosquitoes.

between 5–20 percent, the correct mortality was calculated by applying Abbot's formula (WHO/VBC/1981)

## RESULTS

The results of the larval bio-assay tests carried out with the oil of *C. sativa* have been illustrated in (Fig. 1). The results obtained revealed that the oil of *Cannabis* plant at a concentration of 0.06, 0.1, 0.12 and 0.2 ml/litre of water could induce 100.0 percent mortality in the larvae of *Cx. tritaeniorhynchus*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* respectively. The data obtained were subjected to probit analysis to determine  $LC_{50}$  and  $LC_{90}$  (Table 1). The  $LC_{50}$  and  $LC_{90}$  values estimated for *Cx. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were found to be 0.0101 and 0.0295, 0.026 and 0.0749, 0.0273 and 0.0919, 0.0453 and 0.1803 respectively.

## DISCUSSION

Earlier studies carried out with the ethanolic extracts of *C. sativa* reported that a concentration of 1.5, 2.5 and 4.0 percent could induce 100.0 percent mortality in the larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes respectively (Jalees *et al.*, 1993). The present study revealed that the essential oil of *C. sativa* induced highest mortality at the concentrations of 0.06, 0.1, 0.12 and 0.2 ml/litre of water in the larvae of *Cx. tritaeniorhynchus*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes respectively.

The oil obtained by hydro-distillation was found to be more effective than the ethanolic extraction as the essential oil could induce 100.0 percent mortality at very low concentration as compared to the ethanolic extraction method. It is felt that the

essential oil obtained by hydro-distillation method retains all the active ingredients causing larval mortality, whereas in the essential oil extracted by ethanolic extraction, some of the active ingredients are significantly reduced or lost. In view of the result obtained, it is felt that there is a need to exploit the potential of indigenous plants having insecticidal properties for their eventual use in the control of the arthropods of medical importance as they are safe, biodegradable and eco-friendly as compared to conventional insecticides.

#### ACKNOWLEDGEMENTS

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## Baycidal: Effect on *Tribolium castaneum* Herbst (Coleoptera : Tenebrionidae) Population

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**ABSTRACT:** Batches of 50 : 50 randomly selected 5 days-old untreated virgin males and females of malathion-susceptible (FSS II) and multi-resistant (CTC 12) strains of *Tribolium castaneum* Herbst. were exposed to  $1 \times 10^{-7}$ ,  $5 \times 10^{-7}$  and  $1 \times 10^{-6}$  mg kg<sup>-1</sup> of Baycidal (triflumuron) for 3 and 6 months. All doses of Baycidal controlled the progeny of both strains of the beetles significantly ( $P < 0.001$ ) up to 6 months. The efficacy of Baycidal was found to be dose dependent.

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**KEYWORDS:** Baycidal, Benzoylphenylurea, *Tribolium*, Triflumuron.

### INTRODUCTION

To qualify as grain protectants, compounds should possess a fairly long residual activity to repel and diffuse further attack, or to suppress population development of insects. Benzoylphenyl urea compounds inhibit chitin synthesis in insects, and control growth and development of the treated insects (Grosscurt, 1978). Baycidal and other benzoylphenyl ureas were proved to be effective larvicides (Carter, 1975; Mian and Mulla, 1982a) against lepidopteran, coleopteran, hymenopteran and dipteran insects (Fox, 1990). Baycidal (25% triflumuron, manufactured by Bayer AG) is a promising grain protectant (Mian and Mulla, 1982a,b; Eisa *et al.*, 1984) and found to be safe for the non-target organisms and the environment (Hammann and Sirrenberg, 1980; Fox, 1990). The compound effectively suppressed population growth of stored product insects for 12 months as reported by Mian and Mulla (1982a,b); Dales *et al.* (1994); Elek and Longstaff (1994).

The present study is an attempt to determine the effect of low doses of Baycidal on the F<sub>1</sub> adult progeny of both malathion-susceptible (FSS II) and multi-resistant (CTC 12) strains of *T. castaneum*.

### MATERIALS AND METHODS

Three low doses of Baycidal e.g.  $1 \times 10^{-7}$ ,  $5 \times 10^{-7}$  and  $1 \times 10^{-6}$  mg kg<sup>-1</sup> were chosen for the experiment. Batches of 50 : 50 randomly selected 5 days-old untreated virgin

males and females of both FSS II and CTC 12 strains of *T. castaneum* were kept in 1 lb kilner jars containing 100 g of either treated or untreated media and the top covered by filter paper. To avoid overcrowding, one beetle per gram of food was maintained as suggested by Ogden (1969). After every 30 days additional 100 g of treated or untreated medium was added to each jar to reduce both conditioning the medium and overcrowding. The total number of adults were assessed after three and six months by sieving the medium with a 500 micrometer sieve. Each test was conducted in three replications for both the strains and doses at 30 °C and without controlling light or humidity.

The mean number of adults emerging in the treatment and untreated control was expressed as a percentage by the formula used by Mian and Mulla (1982b):

$$\text{Percent control progeny} = 100(1 - t/c)$$

where

$t$  = number of adults in the treated diet

$c$  = number of adults in the untreated diet

The effects of Baycidal treatments on the progenies of both the strains were tested by the *Kruskal-Wallis test* (Zar, 1974), which is applicable for the samples which do not come from normal populations, and the population variances are heterogenous.

## RESULTS AND DISCUSSION

Table 1 shows that all doses of Baycidal controlled the progeny of both strains of *T. castaneum* significantly up to 6 months. The CTC-12 progeny were less affected than the FSS II after 3 and 6 months exposure. The efficacy of Baycidal was found to be dose dependent. All the three doses controlled the progeny of FSS II (< 90%) for 3 months exposure, whereas 90% control of the CTC-12 progeny for the same exposure period was obtained only at higher concentration ( $1 \times 10^{-6}$  mg kg<sup>-1</sup>).

Diffubenzuron, a related compound effectively inhibited progeny production of *Rhyzopertha dominica*, *T. castaneum*, *T. confusum* and *Sitophilus oryzae* for a long time, when the adults were reared for 3 weeks on treated grains and then transferred to untreated grains (Faragalla *et al.*, 1985). Two weeks of parental exposure to 5 ppm of Baycidal caused a significant reduction in the progeny of all stages of *R. dominica* and *T. castaneum* for up to 8 weeks (Mian and Mulla, 1982a). It is reported that 0.5 and 1.0 ppm of triflumuron (Baycidal) markedly reduced the F<sub>1</sub> progeny yield in red flour beetles, more than diflubenzuron at the same doses (Eisa *et al.*, 1984).

The present results show that Baycidal at very low doses effectively reduced the progeny of both the susceptible and resistant strains of the red flour beetle up to 6 months. The activity of Baycidal in the present study might be due to a combined effect on the adult fecundity, reduced hatching and the mortality of the young larvae as it was reported by Parween (1996). Moreover, the reported ovicidal contact activity of the compound (Mian and Mulla, 1982a; Saxena and Kumar, 1989; Eisa *et al.*, 1984; Saxena and Mathur, 1981) might have inhibited the population build-up.

TABLE 1. Mean number and percentage reduction of  $F_1$  adult progeny of *T. castaneum* recovered after 3 and 6 months parental exposure to Baycidal treated diet.

Dose (mg kg <sup>-1</sup> )	Strain	Mean No. of Progeny after		% reduction of Progeny after	
		3 months	6 months	3 months	6 months
Control	FSS II	2453.67	4957.00	—	—
	CTC 12	1165.33	4964.67	—	—
$1 \times 10^{-7}$	FSS II	169.00	1166.67	93.11**	76.46**
	CTC 12	299.67	2509.00	74.28**	49.46*
$5 \times 10^{-7}$	FSS II	140.33	889.00	94.28**	82.06**
	CTC 12	261.00	1999.33	77.60**	59.72*
$1 \times 10^{-6}$	FSS II	105.00	595.00	95.72**	88.00**
	CTC 12	108.33	611.33	90.70**	87.69**

N.B. \* $P < 0.05$  \*\* $P < 0.001$

Thus not only was it possible to appraise the effects of treatment on parent adults, but the most sensitive stage in the development was exposed routinely to the treatments. The effects of 3 months exposure was very severe on both the strains of *T. castaneum*. At a dose of  $1 \times 10^{-6}$  mg kg<sup>-1</sup> no live larvae or pupae were found, the larvae dyeing in the 3rd and 4th instars. At a dose of  $5 \times 10^{-7}$  mg kg<sup>-1</sup> some live larvae and pupae were obtained, and all the pupae were deformed. Adult mortality in both strains was observed at all dose levels. The mortal effect on the adults was reduced with an increase in exposure time.

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## Population Dynamics of Spiralling Whitefly, *Aleurodicus dispersus* Russell (Aleyrodidae, Homoptera) and its Natural Enemies on Guava in India

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**ABSTRACT:** The spiralling whitefly, *Aleurodicus dispersus* (Russell) (Aleyrodidae, Homoptera) has recently become a pest of guava (*Psidium guajava* L.) in peninsular India. The population of *A. dispersus* was found to be high (164.20 to 218.60 per leaf) from March to June, and low (11.70 to 29.90) from October to January. The density of the whitefly was significantly and positively correlated with maximum and minimum temperature, and negatively correlated with morning and evening relative humidity. The local predators and the rainfall did not show any significant relationship with the population of the spiralling whitefly on guava. The role of temperature and humidity in the regulation of the whitefly was cyclic, and the native predators, which were general, did not have any significant impact on *A. dispersus*. It is suggested to introduce the host specific natural enemies like *Encarsia haitiensis* Dozier and *Nephaspis oculatus* (Wingo) into India for the suppression of the spiralling whitefly.  
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**KEYWORDS:** Population dynamics, Spiralling whitefly, *Aleurodicus dispersus*, predators, guava.

### INTRODUCTION

The spiralling whitefly, *Aleurodicus dispersus* Russell (Aleyrodidae, Homoptera) was first reported in Western ghats of Kerala state in South India, during November, 1993 (Palanisami *et al.*, 1995). Later, it spread to all the five states in peninsular India attacking numerous plant species. The whitefly has become a severe pest of guava (*Psidium guajava* L.) in many locations of South India (David and Regu, 1995; Prathapan, 1996; Mani and Krishnamoorthy, 1996). Several natural enemies were found attacking *A. dispersus* in India (Mani and Krishnamoorthy, 1999). The present study was undertaken to obtain some quantitative data on *A. dispersus* in relation to its natural enemies and weather factors on guava.

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## MATERIALS AND METHODS

Field studies were conducted for a period of two years from July 1996 to June 1998, on guava plants grown in and around Hebbal, Bangalore. These plants were kept free from insecticidal applications during the period of study. Ten plants infested with the spiralling whitefly were chosen for recording observation. Sampling was done at monthly interval on four side shoots (one from each direction) per guava plant. From each shoot, five leaves (6th to 10th leaf from terminal leaf) were collected and brought to the laboratory. The number of nymphs and adults of *A. dispersus* present on each leaf was counted. After counting, the leaves were kept in cloth walled wooden cages (30 × 30 × 30 cm) for recording the emergence of natural enemies, if any, from the whiteflies. Predators were directly counted on four randomly selected shoots of 60 cm length in each plant.

Data on weather parameters, viz., maximum and minimum temperatures (°C), morning and evening relative humidity (%) and rainfall (mm) were collected during the study period. The correlations between the spiralling whitefly and the predators, and the weather factors were calculated to determine their influence on the density of spiralling whitefly. A multiple regression equation of the spiralling whitefly was also fitted with the biotic and abiotic factors.

## RESULTS AND DISCUSSION

The data on the population of spiralling whitefly recorded on guava in different months is presented in Fig. 1. The results revealed the presence of *A. dispersus* throughout the year but the density of the whitefly varied from 11.70 to 218.60 per leaf in different months in the present study. The mean number of the whiteflies per leaf was at 83.30 when the study was initiated in July 1996. Subsequently, the population steadily declined to 11.70 in October 1996. The density of the whitefly remained at low (20.40 to 29.90 per leaf) during September 1996–January 1997. The population of the whitefly began to increase from February, and remained at higher numbers during April–June 1997. The peak population of 218.60 in June was followed by a steep decline to 61.0 in July 1997. Similar trend was also observed during 1997–98.

A total of 13 predators was encountered on *A. dispersus* infesting guava during 1996–98. They included five coccinellids, *Axinoscymnus puttardriahi* Kapur, *Cryptolaemus montrouzieri* Muls., *Chilocorus nigrita* Fab. *Cheilomenus sexmaculata* (Fab.) *Aneglcis cardoni* Wiese, five chrysopids *Apertochrysa* sp., *Mallada astur* Banks, *Mallada boninensis* (Okamoto), *Chrysoperla carnea* (Stephens), *Nobilinus* sp., a nitidulid *Cybocephalus* sp., a drosophilid *Acletoxenus indicus* Malloch and an hemerobid, *Notobiella* sp. Since the population of individual predator species was found in less numbers, they were pooled to determine their influence on the whitefly density. The total mean predator population/plant (4 shoots) was higher (17.60 to 24.00) during July–September 1996 only. In the remaining months of both the years, the density of the predators found to be very low in the present study. No parasitoid was collected on the spiralling whitefly in Bangalore, during the study period.

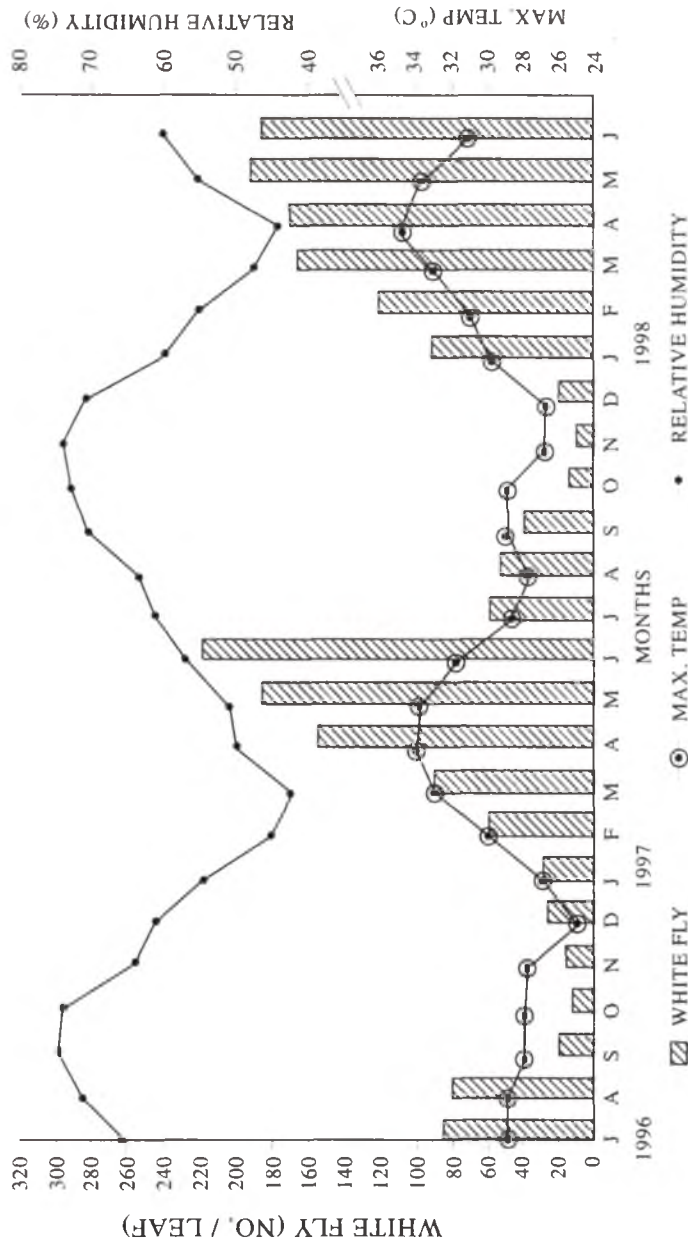


FIGURE 1. Fluctuation in population of spiralling whitefly in relation to temperature and relative humidity on guava during 1996-98.

TABLE 1. Correlations of the spiralling whitefly population with the predators and weather factors on guava at Bangalore

	$X_2$	$X_3$	$X_4$	$X_5$	$X_6$	$Y$ (Whitefly)
$X_1$ (Predators)	-0.156	0.139	0.314	0.387	0.337	-0.099
$X_2$ (Max. temp.)		0.389	-0.771**	-0.780**	-0.255	0.860**
$X_3$ (Min. temp.)			0.059	0.070	0.414*	0.433*
$X_4$ (Morning humidity)				0.981	0.569**	-0.630**
$X_5$ (Evening humidity)				0.577*	-0.646**	
$X_6$ (Rainfall)					-0.354	

\*Significant ( $P = 0.05$ ); \*\*High significant ( $P = 0.01$ ).

Among the predators, *A. puttarudriahi*, *C. montrouzieri* and *M. astur* were found more frequently than the others. The activity of *A. puttarudriahi* was limited only for three months viz., July–September in both the years. An highest population of 22.85 per plant (4 shoots) was recorded in July 1996, but the predator was found in negligible numbers in July 1997. *C. montrouzieri* was found associated with *A. dispersus* throughout the year, but the predatory population remained in lower numbers in both the years. A maximum of 3.10 *Cryptolaenus* larvae/plant was observed in August 1998. The activity of the chrysopids was more in May–June, and a maximum of 2.75 predators/plant was observed during this period.

The relationship of the population of spiralling whitefly with its predators and weather parameters was studied using correlation coefficients (Table 1). The maximum and minimum temperature showed positive correlation with the whitefly population, but the relationship between maximum temperature and the whitefly was found highly significant ( $r = 0.860$ ). The trend in the population of spiralling whitefly in relation to maximum temperature is shown in Fig. 1. The presence of higher population during March–June might be due to higher temperature (29 to 35 °C) which might have probably helped in faster multiplication of the whitefly. In Hawaii also, significant positive correlation was obtained between the temperature and spiralling whitefly abundance (Kumashiro *et al.*, 1983). According to Waterhouse and Norris (1989), the population of *A. dispersus* rose in warmer and dry weather in Hawaii. Ranjith *et al.* (1996) also reported that the spiralling whitefly increased drastically in summer months (March–May) in Kerala. In the present study, the population of *A. dispersus* was found to be low in winter months (October–January) in which the minimum temperature ranged from 13° to 21 °C ( $r = 0.413$ ). In Hawaii also, cooler temperatures resulted in temporary reduction in the population of *A. dispersus* (Waterhouse and Norris, 1989).

The trend in the whitefly population in relation to relative humidity (average of morning and evening relative humidity) is given in Fig. 1. Both morning relative humidity ( $r = -0.630$ ) and evening relative humidity ( $r = -0.646$ ) had negative

TABLE 2. Regression of predators and weather factors on spiralling whitefly

Variable	Regression coefficient	Standard Error	<i>t</i> -value	<i>P</i> -value
Predators	0.570	1.450	0.393	0.699
Max. temp.	23.498*	8.752	2.685	0.016
Min. temp.	1.687	5.368	0.314	0.757
Morning RH	-1.245	5.863	-0.212	0.834
Evening RH	1.842	4.367	0.422	0.678
Rainfall	-0.394	0.178	-2.017	0.040

\*Significant at  $P = 0.05$ .

and significant influence on the population of the whitefly in the present study. The presence of the whitefly population at low level during October–January might also be due to high humidity (70–79%) besides the low temperature in this period. This is in conformity with Chandrasekara (1990) who had also reported significant influence of relative humidity on the density of spiralling whitefly on guava in Sri Lanka.

Heavy sporadic rains resulted in temporary reduction in the population of spiralling whitefly in Hawaii (Waterhouse and Norris, 1989). In lowland Honolulu, the whitefly density was negatively correlated with the rainfall but in highland Honolulu, monthly rainfall fluctuated throughout the year, and there was no significant correlation between the whitefly density and the rainfall. In the present study, there was negative relationship between the rainfall and the whitefly population, but the influence was not found to be significant ( $r = -0.354$ ). According to Ranjith *et al.* (1996), there was decrease in the whitefly population after the pre-monsoon showers in South India. The multiple regression equation fitted with the predators and weather parameters to predict the whitefly population ( $Y$ ) was

$$Y = -64.650 + 0.570X_1 + 23.498X_2 + 1.687X_3 - 1.245X_4 + 1.842X_5 - 0.394X_6$$

with  $R^2 = 0.731$ , where  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_5$  and  $X_6$  denote the total predators, maximum temperature, minimum temperature, morning relative, humidity evening relative humidity and the rainfall, respectively.

The partial regression coefficient of the maximum temperature was found to be significant. But the partial regression coefficients of minimum temperature, morning and evening relative humidity and rainfall were found to be non significant (Table 2).

In Indonesia, the native predators were incapable of preventing the outbreaks of spiralling whitefly on guava (Kajita *et al.*, 1991). In the present study also, neither the correlation between the native predators and the whitefly nor the partial regression coefficient of the predators was found to be significant. The local predators recorded in the present study are general, and the lack of each individual species in sufficient numbers might be attributed to their feeding habits not showing a definite preference for spiralling whitefly. However, in Hawaii, there was significant correlations between the abundance of spiralling whitefly and the introduced natural enemies chiefly the parasitoid, *Encarsia haitiensis* Dozier and the coccinellid predator *Nephaspis oculatus*

(Wingo) (Kumashiro *et al.*, 1983). These two natural enemies played the dominant role in suppression of whitefly in Hawaii and also in many other countries.

Although, the abiotic factors like temperature and relative humidity might have significant effect on the population of whitefly, their regulatory role is cyclic in every year. For the permanent suppression of the spiralling whitefly, it is suggested to introduce the host specific natural enemies, *E. haitiensis* and *N. oculatus* into India.

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## Seasonal Variations in the Energy Contents of *Cybister confusus* Sharp (Coleoptera : Dytiscidae) of a Fish Pond

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**ABSTRACT:** The paper describes the calorific values of water diving beetle (*Cybister confusus* Sharp) of a fish farming pond. The maximum calorific values of the beetles  $5.739 \pm 0.005$  Kcal/gram dry weight and  $5.97 \pm 0.001$  Kcal/gram ash free dry weight was recorded in the month of January. The minimum values  $4.951 \pm 0.004$  Kcal/gram ash free dry weight was recorded in September. It was observed that the winter and summer months have relatively more values of calorific contents than the remaining months of the year. © 2000 Association for Advancement of Entomology

**KEYWORDS:** Seasonal Variations, Calorific Values, *Cybister confusus* Sharp.

### INTRODUCTION

Considerable work has been done on the estimation of the energy contents of freshwater animals (Prus, 1970; Slobodkin and Richman, 1961; Jana and Pal, 1981; Roy and Datta Munshi, 1983) but not information is available on the calorific values of animals occupying at higher trophic levels of food chains of freshwater ecosystems. *Cybister confusus* Sharp, commonly known as predaceous diving beetle is a top carnivore, most frequently encountered in freshwater habitats. The purpose of this study is to provide data on energy contents of predatory aquatic insects most abundant in the fish farming ponds and are injurious to fish spawn, fry and fingerlings of major carps.

### MATERIALS AND METHODS

*Cybister confusus* Sharp was collected from local fish ponds by aquatic insect collecting net during the period January to December 1996 and brought to the laboratory. The beetle was placed in an oven for several hours at 60 °C till constant weight is obtained. The loss in weight during drying was taken as the weight of water. The sample was homogenized in a mortar and the powder is pestled with distilled water to form pellets of approximately one gram under a pellet forming machine.

The pellets were dried again in the oven for at least 24 hours before burning in an oxygen bomb calorimeter. Five replicated determinations were made for each sample. In drying process of the material precaution was taken not to overheat the samples.

The maximum temperature difference was obtained in 6–7 minutes after the explosion. The ash contents in the samples were determined by weighing the residual in the cup after burning. Before pressurization the bomb was flushed with oxygen (3 times) to remove nitrogen from the bomb. Taking into account the ash content of the sample, the ash-free energy values of the material was calculated.

The energy contents of the sample was determined according to the following formula derived from the heat balance equation (Gorecki, 1975).

$$W_g = \frac{W_w(t_n + C - t_0) - \sum b}{G}$$

where,

- $W_g$  = is the energy contents of the sample,
- $W_w$  = is the net calorific value of the calorimeter system, i.e. water equivalent of the calorimeter = 2390,
- $t_n$  = the final temperature of the central period,
- $t_0$  = the initial temperature of the central period,
- $C$  = Correction for radiation = 0.079,
- $\sum b$  = the sum of the correction = 20,
- $G$  = Weight of the sample in grams.

## RESULTS AND DISCUSSION

The seasonal changes in the energy contents of the *Cybister confusus* Sharp have been shown in Table 1. This aquatic beetle is predaceous on a variety of small animals including small fishes, fingerlings and tadpoles of the freshwater habitats. It was investigated that the maximum calorific values ( $5.739 \pm 0.005$  Kcal/gram dry weight and  $5.697 \pm 0.001$  Kcal/gram ash free dry weight) was recorded in the month of January. The minimum calorific values ( $4.951 \pm 0.044$  Kcal/gram dry weight and  $4.901 \pm 0.028$  Kcal/gram ash free dry weight) was recorded in September. The percentage ash fraction varied from  $0.097 \pm 0.006$  to  $0.039 \pm 0.006$  with maximum and minimum values in April and December respectively (Table 1).

The energy contents of this predatory aquatic beetle varies from season to season and from month to month. The availability of proper food during the different months of the year affects the calorific contents of this insect. Since this beetle attack only moving prey, therefore, abundance and dominance of food in the habitat also matter much in the caloric contents of this species. The significant variation in the energy contents of this beetle was also influenced by the caloric equivalent of the daily ration available, which account for this seasonal variation.

It was found that in this beetle the changes in the energy contents were effected

TABLE 1. Seasonal variations in calorific values of *Cybister confusus* Sharp

Months year (1996)	Calorific values (Kcal/g dry wt.)	Ash fraction (% dry wt.)	Organic fraction (Kcal/g ash free dry wt.)
Jan	5.739 $\pm$ 0.005	0.042 $\pm$ 0.005	5.697 $\pm$ 0.001
Feb	5.143 $\pm$ 0.061	0.041 $\pm$ 0.001	5.101 $\pm$ 0.012
Mar	5.308 $\pm$ 0.049	0.044 $\pm$ 0.002	5.265 $\pm$ 0.026
Apr	5.407 $\pm$ 0.005	0.047 $\pm$ 0.006	5.359 $\pm$ 0.035
May	4.950 $\pm$ 0.044	0.061 $\pm$ 0.004	4.901 $\pm$ 0.028
Jun	5.341 $\pm$ 0.006	0.063 $\pm$ 0.003	5.279 $\pm$ 0.027
Jul	5.299 $\pm$ 0.007	0.056 $\pm$ 0.007	5.243 $\pm$ 0.024
Aug	5.154 $\pm$ 0.006	0.051 $\pm$ 0.005	5.103 $\pm$ 0.013
Sep	5.273 $\pm$ 0.005	0.052 $\pm$ 0.007	5.244 $\pm$ 0.024
Oct	5.260 $\pm$ 0.004	0.043 $\pm$ 0.008	5.227 $\pm$ 0.022
Nov	5.227 $\pm$ 0.005	0.045 $\pm$ 0.001	5.181 $\pm$ 0.018
Dec	5.053 $\pm$ 0.009	0.039 $\pm$ 0.006	5.014 $\pm$ 0.010

largely through variation in the relative size of the ash-fraction. However, the caloric contents of the organic fraction remained relatively more or less constant. Schindler *et al.* (1971) have reported that the changes in the caloric contents of the organic fraction may reflect changes in the amount of lipid contents. The calorific values of the animals depend on the relative size of the ash-fraction, age, sex, developmental stages as well as the fat content of the body (Golley, 1960; Rodgers and Qadri, 1977). Thus, the energy contents of an insect is related to a considerable degree on the fat content and to a lesser extent on the mineral content of the body. The fat content of the individual varies throughout the life and this may be the explanation of the seasonal and monthly changes in the calorific values of the aquatic beetle in the present study (Kumar, 1987).

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## Volatile Constituents of Metathoracic Scent Secretions of Adult *Cyclopelta siccifolia* Westwood (Hemiptera : Pentatomidae)

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**ABSTRACT:** Spectra of twelve compounds from the metathoracic scent glands of adult *Cyclopelta siccifolia* were analysed by gas chromatography and mass spectrometry (GS-MS); and six compounds were identified as trans-hex-2-enal, cis-4-oxo-2-hexanol, acetonyl acetone, N-N-dimethyl acetoacetamide, n-butyl isocyanate and isobutyl chloroformate. © 2000 Association for Advancement of Entomology

**KEYWORDS:** *Cyclopelta siccifolia*, metathoracic scent glands, scent secretion.

### INTRODUCTION

Adult pentatomid bugs are remarkably distinct for the release of noxious exocrine secretions from the metathoracic scent glands whenever they are distributed or irritated. These secretions are highly volatile and are usually emitted as droplet or as spray (Valcurone Dazzine and Vita Finizi, 1974). These secretions contain a dazzling array of chemical compounds (Aldrich, 1988; Aldrich *et al.*, 1987, 1984, 1982; Blum, 1981, 1978; Cmelik, 1969; Gilby and Waterhouse, 1965; Janaiah, 1993; Staddon and Olagbemiro, 1984; Surender and Janaiah, 1990; Vidya Sagar *et al.*, 1998). Scent secretions may be defensive irritants (Blum, 1961; Janaiah, 1993) sex-attractant (Park and Sutherland, 1962), alarming (Levinson and Barilan, 1971) antirespiratory (Canuto *et al.*, 1985), antimitotic (Pundhir *et al.*, 1966), antimicrobial (Vidya Sagar *et al.*, 1997), carcinostatic (Schauenstein *et al.*, 1987) and neurotoxic (Escoubas and Nakajima, 1994).

The present investigation deals with the structure of metathoracic scent glands and chemical analysis of the scent secretions of adult *Cyclopelta siccifolia*.

### MATERIALS AND METHODS

Fifteen days old adult bugs of *Cyclopelta siccifolia* were collected from the host plant *Sesbania sepiciosa* (Fabaceae) in the betal gardens and also from the intermediate

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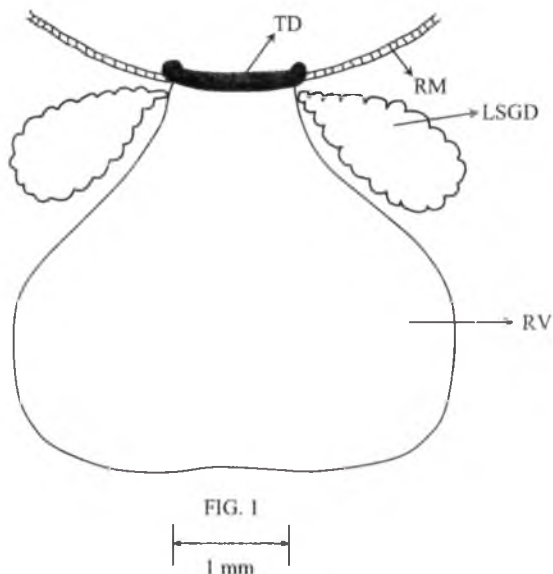


FIGURE 1. Metathoracic Scent gland in adult *Cyclopelta siccifolia* (dorsal view). TD = Transverse duct; RM = Regulatory muscle; LSGD = Lateral or accessory scent gland; RV = Reservoir.

host plant *Pongamia pinnata* (Fabaceae) at Bhuvanagiri, Nalgonda district, Andhra Pradesh. They were maintained in the laboratory on the cut leaves of the host plant at room temperature (30 °C) for a week in the laboratory. The scent secretions were collected and pooled from the metathoracic scent glands of 50 adults by placing microcapillaries against opening of the gland.

The pooled secretion were analysed by gas chromatography and mass spectrometry (GC-MS) and the compounds were identified with the help of authentic samples obtained from Aldrich Chemical Company, Milwaukee, U.S.A. GC-MC analysis was conducted by using a Finnigan MAT quadrupole 1020B Mass Spectrometer with Hewlett Packard high performance capillary column. Unknown and authentic samples (0.5  $\mu$ l) were injected through sample boat separately. The column was held at 60 °C/m in, and samples were programmed for 30 minutes. All the spectra were run at 70 eV at manifold temperature of 70 °C.

## RESULTS

In adult *C. siccifolia* the metathoracic scent gland complex is made up of a brick red median ventral reservoir with a pair of lateral or accessory glands and a pair of regulatory muscles (Fig. 1).

TABLE I. Volatile Scent constituents of metathoracic scent glands of adult *Cyclopelta siccifolia* with GC-MS evidence

Peak No.	Component	Molecular weight	Masses of ions in order of abundance
1	<i>trans</i> -hex-2-enal	98	M <sup>+</sup> 98, base peak 41 (base peak) 55, 56, 83 and 98 (molecular ion)
2	<i>cis</i> -4-oxo-2-hexanol	112	M <sup>+</sup> 112, base peak 55, 45, 55 (base peak) 57, 73, 83 and 112 (molecular ion)
3	Unidentified	83	M <sup>+</sup> 83, base peak 57, 45, 57 (base peak) 73 and 83 (molecular ion)
4	Acetonyl acetone	114	M <sup>+</sup> 114, base peak 42, 41, 42 (base peak), 73, 87, 89, 123 and 129 (molecular ion)
5	N-N-dimethyl acetoacetamide	129	M <sup>+</sup> 129, base peak 57, 43, 57 (base peak), 73, 87, 89, 123 and 129 (Molecular ion)
6	Unidentified	133	M <sup>+</sup> 133, base peak 75, 43, 55, 59, 75 (base peak) 87 and 113 (molecular ion)
7	Unidentified	127	M <sup>+</sup> 127, base peak 59, 47, 59 (base peak), 75, 87, 99 and 127 (molecular ion)
8	n-butyl isocyanate	99	M <sup>+</sup> 99, base peak 57, 43, 57 (base peak), 71, 85, 89, 99 (molecular ion)
9	Isobutyl chloroformate	136	M <sup>+</sup> 131, base peak 57, 43, 57 (base peak), 79, 89, 99, 123 and 136 (molecular ion)
10	Unidentified	181	M <sup>+</sup> 181, base peak 55, 41, 55 (base peak), 81, 97, 109, 123, 181 (molecular ion)
11	Unidentified	153	M <sup>+</sup> 153, base peak 57, 41, 57 (base peak), 69, 89, 111, 153 (molecular ion)
12	Unidentified	195	M <sup>+</sup> 195, base peak 95, 41, 55, 67, 95 (base peak) 109, 125, 167 and 195 (molecular ion)

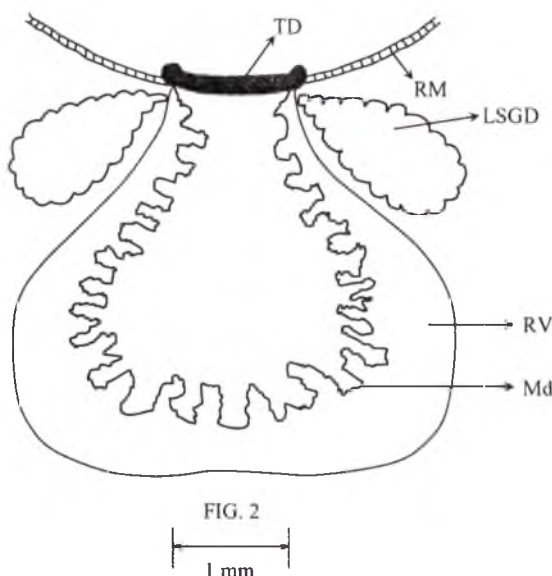


FIGURE 2. Metathoracic Scent gland in adult *Cyclopelta siccifolia* (ventral view). Md = Median Collecting duct; Rv = Reservoir.

The scent gland reservoir is present midventrally in the metathoracic region, below the nerve cord, and this may reach upto the second abdominal segment when bug is fully matured. The medium scent duct runs zig-zag like a necklace all along the ventral margin of the reservoir (Fig. 2). On the lateral side of the reservoir a pair of colourless lobulated and tubular lateral glands or accessory glands are present. The lateral glands open into the reservoir through a transverse duct, which opens outside ventrally by a pair of ostioles, located at the base of the coxae of the third pair of legs. A pair of regulatory muscles are attached to the lateral sides of the reservoir to operate the glands.

The GC-MS analysis of the scent secretion of the metathoracic scent glands of the adult *C. siccifolia* showed a chromatogram consisting of mainly 12 peaks (Fig. 3) (Table 1).

**Peak 1** showed molecular ion  $m/z$  98 and the base  $m/z$  41. It showed other prominent peaks at  $m/z$  55, 56 and 83. The other fragmentation pattern clearly suggests that the component is trans-hex-2-enal.

**Peak 2** showed the molecular ion  $m/z$  112 with base peak at 55. The base peak of this spectrum results with the loss of propanoyl group from molecular ion. The compound was identified as cis-4-oxo-2-hexenol.

**Peak 3** Unidentified.

**Peak 4** showed the molecular ion at 114 with poor intensity and the base peak appeared at  $m/z$  42. The base peak is resulted by the loss of methyl and acetyl

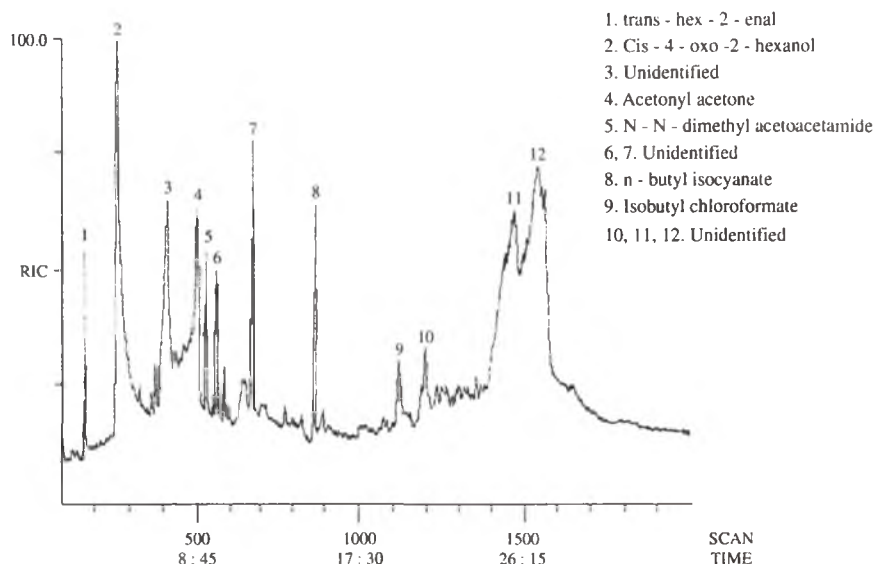


FIGURE 3. Gas-chromatographic separation of metathoracic scent gland secretions of adult *Cyclopelta siccifolia* with GC-MS evidence.

groups from molecular ion resulting in Ketene structure confirms that it is acetonyl acetone.

**Peak 5** showed the mass spectrum with the molecular ion  $m/z$  129 and the base peak 57. The base peak results with the loss of  $\text{CH}_3\text{COCH}_2$  fragment from molecular ion. The compound was confirmed as N-N-dimethyl acetoacetamide.

**Peak 6 and 7** Unidentified.

**Peak 8** showed molecular ion  $m/z$  99 and the base peak 57. The base peak of the spectrum is due to n-butyl cation, which confirms that it is n-butyl isocyanate.

**Peak 9** showed molecular ion at  $m/z$  136 with base peak  $m/z$  57. The base peak of the spectrum is due to isobutyl cation. The compound could be isobutyl chloroformate.

**Peak 10, 11 and 12** Unidentified.

Cis-4-oxo-2-hexenol, Acetonyl acetone, N-N-dimethyl acetoacetamide, n-butyl isocyanate and isobutyl chloroformate are detected for the first time in a phytophagous pentatomid bug, in this investigation.

## DISCUSSION

The characteristic stink of the pentatomid bugs are due to the enrichment of mixture of aldehydes (Waterhouse *et al.*, 1961; Choudhuri and Das, 1968; Janaiah, 1993). In the present investigation, the pungency of the scent is mostly due to the presence of aldehydes like trans-hex-2-enal and acetonyl acetone. These two components acted

as repellents to birds and other vertebrate predators. Rejection of bugs by birds is basically based on the taste (Schlee, 1986). Generally the combination of trans-4-oxo-enal and trans-hex-2-enal found in almost all the immature heteropterans, functions as alarm pheromone. Rengnier and Wilson (1971) called these as super hormones. In *C. siccifolia*, trans-hex-2-enal is the major component which is defensive in function.

Mentathoracic scent secretions of *C. siccifolia* are highly irritative to mucous membrane of nostrils and are corrosive in nature. If the scent is contacted to human skin, it causes burning sensation followed by the formation of blisters which remained for a few hours and left a yellow stain on the skin. The blisters remained for 4 to 5 days and subsequently the skin is peeled off. The corrosiveness is mainly due to acetyl acetate and N-N-dimethyl acetoacetamide. Similar observations were reported with the metathoracic scent secretions of *Tessaratoma javanica* (Janaiah *et al.*, 1979), *Halys dentatus* (Surender and Janaiah, 1990) and *Aspongopus* sp. (Ravinder, 1997).

Isobutyl chloroformate is an ester found in the metathoracic scent secretions of *C. siccifolia*. Generally esters are sex-attractants (Menker, 1960; Aldrich *et al.*, 1987) trans-hex-2-enyl acetate, an ester which is reported in *Chrysocoris purpureus* (Leela Kumari *et al.*, 1984) and in *T. javanica* (Janaiah *et al.*, 1979) has no sexual role but it is primarily defensive in function. In pond skater, *Gerris spinole* the esters are neither a sex-pheromone nor defensive but these are used as hydrofuge substance, which is essentially needed for skating on the water surface (Ramamurthy and Krishnanandam, 1967).

Acetyl acetone is not only responsible for pungency of the scent but it also acts as a non-specific contact poison and effective against a broad spectrum of vertebrate and invertebrate predators (Eisner *et al.*, 1970).

N-butyl-isocyanate is a cyanate compound. It is highly poisonous and is the best chemical for defense against number of invertebrates, birds and other vertebrate predators. It is more toxic or deterrent out of all components of the metathoracic scent secretions of *C. siccifolia*.

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## Studies on Ants (Formicidae) of Rajasthan—II Dungarpur

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**ABSTRACT:** Ants were collected from various localities of Dungarpur district from ground, herbaceous cover and trees.

Nine species of Formicidae were collected from different localities of Dungarpur pertaining to six subfamilies viz., Ponerinae, Aenictinae, Dolichoderinae, Formicinae, Pseudo-myrmicinae and Myrmicinae. Out of nine species five are new records from Rajasthan. A zoogeographical analysis has indicated that five species are Universal, two restricted to Indian sub-continent and two species are endemic to India.

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**KEYWORDS:** Ants, Dungarpur, Rajasthan.

### INTRODUCTION

The author in her first paper on series of Rajasthan ants dealt with the ants of Jodhpur. This is the second paper on series of Rajasthan ants in which ants of Dungarpur region are reported.

The district Dungarpur (dungar-hills) is situated in the southern part of Rajasthan and lies between 23° 20' and 24° 01'N and 73° 22' and 74° 23'E. It is bounded on the north by Udaipur district and on the east by Banswara district. On its south and west, it has a common border with the state of Gujarat.

Its maximum span from north to south is about 74 kms and from east to west is about 104 kms.

The district though fairly open in the south and east is interspersed with stony hills covered with low jungle of cactus, jujube trees and salar (*Baswellia serrata*) a gum producing tree with several varieties of shrubs and trees requiring neither a deep soil nor a high soil moisture regime. Thordenda (*Euphorbia niyulia*) are found everywhere. Probably because of the rich vegetation core in the district, a number of ant species are found but very little work has been carried out on the ant fauna in northwest India.

Bingham's (1903) Fauna is the main source of knowledge on ants of India. Rothney (1889) published extensive notes on ants of Bengal and Wroughton (1892) provided a good account on ants of Maharashtra. Chapman and Capco (1951) published a checklist of the ants of Asia. Chhotani and Ray (1976) described the hymenopterous

fauna of Rajasthan and dealt with a few species of desert region. Chhotani and Maiti (1977) worked on the ants of Andaman Islands. Pajni and Suri (1978) reported the Formicid fauna of Chandigarh. Roonwal (1975) discussed the plant pest status of *Dorylus orientalis*. Mathew (1980, 1983, 1984) dealt with the ants of Northeast India. Sharma (1981) gave a short note on the ants of Desert area. Ali (1991) reported the ants fauna of Karnataka. The author prepared a paper Studies on Ants of Rajasthan—I. Jodhpur (1995) and reported some species of ants of Jodhpur region. Bolton (1995) has dealt with the taxonomic and zoogeographical census of the extant and taxa (Hymenoptera : Formicidae).

Ants were collected from various localities of Dungarpur district from ground, herbaceous cover and trees.

Nine species of Formicidae were collected from different localities of Dungarpur pertaining to six subfamilies viz., Ponerinae, Aenictinae, Dolichoderinae, Formicinae, Pseudo-myrmicinae and Myrmicinae. Out of nine species five are new records from Rajasthan. A zoogeographical analysis has indicated that five species are Universal, two restricted to Indian sub-continent and two species are endemic to India.

Abbreviations used

N.S. Rathore—N.S.R.

Ants are polymorphic social insects having three distinct forms—The perfect and fertile female (♀), the male (♂) and a worker (♂ major or minor). The largest forms of workers are soldiers.

Identification key is based on the worker caste (♂) of an ant.

Key to the sub-families of Family Formicidae.

I. Pedicel of the abdomen one or two jointed.

1. A more or less marked constriction between basal two segments of abdomen. Pedicel one jointed. ...Sub-family Ponerinae
2. No constriction between basal two segments of abdomen.
  - (i) Anal aperture in form of a transverse slit.
    - (a) Eyes never present, blind, Pedicel two jointed. ...Sub-family Aenictinae
    - (b) Eyes always present  
Pedicel one jointed ...Sub-family Dolichoderinae
  - (ii) Anal aperture circular  
Pedicel one jointed ...Sub-family Formicinae

II. Pedicel of the abdomen two jointed.

- (i) Pedicel remarkably elongate, generally the anterior node is elongately petiolate, sometimes the posterior node is also petiolate, giving great flexibility to the abdomen ...Sub-family Pseudomyrmicinae
- (ii) Pedicel not so elongate, the anterior node with a long petiole anteriorly and a short petiole posteriorly. ...Sub-family Myrmicinae

### I. Sub-family Ponerinae Lepeletier

Tribe Ponerini Forel

*LEPTOGENYS* Roger

*Leptogenys (Lobopelta) processionalis* (Jerdon)

*Leptogenys (Lobopelta) processionalis* (Jerdon) *Madras Jour. Lit. Sc.* 17 (1951) 118,  
♀ (*Ponera*)

*Material:*

12 exs, N.S.R., 21.8.84

Collected from ground.

*Diagnostic characters:*

Length – ♀ – 8–9 mm.

Dark castaneous brown, smooth, polished and shining. Clypeus narrow not dentate. Mandibles broad, longitudinally striate armed at the apex with four unequal teeth and denticulate along the inner margin. Antennae short, scape just passing the top of the head. Thorax slightly narrower than the head, pro-meso and meso-metanotal sutures deeply marked. Claws pectinate. Node of the pedicel convex in front, flat posteriorly.

*Distribution:* Rajasthan—Dungarpur.

*Range:* Karnataka, Tamil Nadu, Kerala, Madras, Travancore, Sri-Lanka.

*Remarks:* Recorded for the first time from Rajasthan.

*Leptogenys ocellifera* is the only Ponerine species with mandibles armed at the apex with four unequal teeth and denticulate along the inner margin.

### II. Sub-family Aenictinae

Tribe Aenictini

*AENICTUS* Shuck

*Aenictus (Aenictus) brevicornis* (Mayr)

*Aenictus (Aenictus) brevicornis* (Mayr)

(*Typhalatta*), *Verh. Zool. bot. Ges. Wien*, 28 (1878): 668 & 669, ♀ : Forel, *J. Bombay nat. Hist. Soc.*, 13 (1901), p. 446, ♀.

*Material:*

8 exs, N.S.R., 21.8.84

Ants collected from aak plant and soil underneath.

*Diagnostic characters:*

Length ♀ - 2.5–3 mm.

Reddish yellow, head smooth and shining, thorax only sculptured. Mandibles with three distinct teeth. Scape of antennae very short, about half length of head. Thorax narrower than the head, pronotum convex, smooth and shining, basal portion of the metanotum passing by a gradual curve. Nodes of pedicel rounded, shining. Abdomen elongate, oval.

*Distribution:* Rajasthan—Dungarpur

*Range:* India—N.W. Provinces. Chandigarh, Assam, West Bengal, Calcutta, Karnataka, Kerala.

*Remarks:* This species has been recorded for the first time from Rajasthan.

### III. Sub-family Dolichoderinae Forel

Tribe Dolichoderini Emery

*TAPINOMA* Forster

*Tapinoma (Micromyrma) melanocephalum* (Fabr.)

*Tapinoma (Micromyrma) melanocephalum* (Fabr.)

(*Formica*), *Ent. Syst.* ii (1796), p. 353; Forel, *J. Bombay nat. Hist. Soc.* 9 (1895) 472, ♀.

*Material:*

6 exs, N.S.R., 21.8.84

Ants collected under stone

*Diagnostic characters:*

Length ♀ – 1.5 mm.

Head and thorax dark brownish in colour, abdomen yellowish white. Mandibles triangular, broad with the masticatory margin equal to the outer margin and armed with numerous teeth. Antennae long, the scape extending beyond the top of the head. Thorax viewed from the side not emarginate, the pro-meso and meso-metanotal sutures distinct. Pedicel with a distinct node. Base of the abdomen overhanging the pedicel.

*Distribution:* Rajasthan—Dungarpur.

*Range:* Whole continent of India. Spread throughout the tropics of both the hemispheres.

### IV. Sub-family Formicinae Latreille

(ii) Tribe Camponotini Forel

*CAMPONOTUS* Mayr

*Camponotus (Tanaemyrmex) compressus* (Fabricius)

*Camponotus (compressus)* (Fabr.) (*Formica*), *Mant. Ins.* i (1787): 307 ♀ : Smith (*Formica*), *Cat.* 6 (1858): 13, ♂ ♀

*Camponotus maculatus*, Fabr., race *compressus* (Fabr.), Forel, *J. Bombay nat. Hist. Soc.* 7 (1892) : 239 & 240 *Camponotus (Tanaemyrmex) compressus* : Emery, C., *Gen. Insect.*, Fasc. 183 (1925) : 98.

*Material:*

2 exs, N.S.R., 20.8.84

Ants collected from Babul tree (*Acacia nilotica*) and under stone.

*Diagnostic characters:*

Length ♀ maj. - 13 mm.

Black opaque. Maxillary palpi 6 jointed. Mandibles with seven teeth. Antennae 12-jointed and inserted at a perceptible distance from posterior margin of clypeus. Head

not truncate anteriorly. Thorax viewed from side forming a regular arch. Thorax and node of pedicel without spines. Tibia of the legs prismatic.

*Distribution:* Rajasthan—Dungarpur, Jodhpur, Gudha, Phulera.

*Range:* Throughout India. Sri-Lanka, Myanmar, Russia, Arabia and Africa.

*CAMPONOTUS* Mayr

*Camponotus (Orthonotomyrmex) sericeus* (Fabr.)

*Camponotus (Orthonotomyrmex) sericeus* (Fabr.) *Ent. Syst, Suppl.* 1798 : 279; Forel, *J. Bombay nat. Hist. Soc.* 7 (1892) : 223 & 231, ♀.

*Material:*

2 exs, N.S.R., 22.8.84

Ants under stone.

*Diagnostic characters:*

Length ♀ maj. - 10 mm.

Black opaque, Maxillary palpi 6-jointed. Mandibles with five teeth. Antennae 12-jointed and inserted at a perceptible distance from posterior margin of clypeus. Head not truncate anteriorly. Regular arch of the thorax interrupted at the meso-metanotal suture by the metanotum forming an angle with the mesonotum, basal portion of metanotum horizontal. Thorax and node of pedicel without spines. Tibia of legs spinous beneath, node of pedicel thick, globose.

*Distribution:* Rajasthan—Dungarpur

*Range:* Common throughout our limits, Chandigarh, Karnataka, Myanmar, Sri-Lanka, Africa.

*Remarks:* Recorded for the first time from Rajasthan. According to Bingham (1903), the specimens found in Myanmar and Sri-Lanka have the head blood red in colour but the head of the specimens collected here is also dull red.

(ii) Tribe Plagiolepidini Forel

*PLAGIOLEPIS* Mayr

*Plagiolepis jerdoni* Forel

*Plagiolepis jerdoni*, Forel, *J. Bombay nat. Hist. Soc.*, 8 (1894): 415 & 416, ♀.

*Material:*

10 exs, N.S.R., 20.8.84

Ants collected from plants.

*Diagnostic characters:*

Length ♀ - 1.5 mm.

Brownish black, head smooth, polished and shining. Frontal area distinct. Maxillary palpi 6-jointed. Mandibles narrow, masticatory margin with five teeth, the apical tooth long and acute. Antennae 11-jointed, slender, extending slightly more beyond the top of the head. Thorax short and broad, pronotum large, convex, mesonotum from above circular and convex, meso-metanotal emargination well marked. Metanotum and node of pedicel without spines. Node of the pedicel low, strongly inclined to the front.

*Distribution:* Rajasthan—Dungarpur.

*Range:* Western India, Karnataka, Kerala, Poona distt.—Kanara, Travancore.

*Remarks:* Occurs in Western India but recorded for the first time from Rajasthan.

### V. Sub-family Pseudomyrmicinae Emery

Tribe Pseudomyrmicini Forel

*TETRAPONERA* Smith

*Tetraponera* (*Tetraponera*) *rufonigra* (Jerdon)

*Tetraponera* (*Tetraponera*) *rufonigra* (Jerdon) (*Eciton*), *Madr. Jour. Lit. Sc.* 12 (1851) : III; id. *A. M. N. H.* (2) 13 (1854); 33.

*Tetraponera* (*Tetraponera*) *rufonigra* Emery, *C. Gen. Insect., Fasc.*, 174 A : (1921) : 23.

*Material:*

5 exs., N.S.R., 20.8.84; 6 exs, N.S.R., 21.8.84

*Diagnostic characters:*

Length ♀ - 12 mm.

Head, 2nd joint of pedicel black the mandibles, antennae, thorax and 1st joint of the pedicel more or less red. Posterior margin of clypeus not produced between bases of antennae. Mandible with 6 teeth, more or less linear. Antennae 12-jointed, short Ocelli present. Thorax elongate, the pronotum broad, its anterior lateral angles dentate, a medial small longitudinal tubercle at its posterior margin, pro-mesonotal suture arched to the front, mesonotum small, flat, a deep emargination at the meso-metanotal suture. Metanotum longer than the pro and meso together. Pedicel elongate, the 1st node oval with a long petiole, 2nd node conical with a short petiole.

*Distribution:* Rajasthan—Dungarpur, Jodhpur.

*Range:* Throughout India, Sri-Lanka, Myanmar, China, singapore, Cambodia.

### VI. Sub-family Myrmicinae Lepeletier

#### 1. Tribe Meranoplini Emery

*MERANOPLUS* Fred Smith

*Meranoplus bicolor* (Guérin)

*Meranoplus bicolor*, Guér. (*Cryptocerus*) *Cuv. Iconogr. Regne Anim. Ins.* 7 (1845) : 425; Smith, *Trans. Ent. Soc. Lond.* 1875 (34), pl. 1. figs 1-3, ♀, ♀, ♂.

*Meranoplus bicolor* Emery, *C., Gen. Insect, Fasc.* 174 B(1923) : 228.

*Material:*

1 ex, N.S.R., 22.8.84; 3 exs, N.S.R., 21.8.84

Ants collected by sweeping and understone.

*Diagnostic characters:*

Length ♀ - 4.8 mm.

Bright ferruginous red with abdomen black. Pilosity very long soft abundant and of grey colour. Mandibles narrow, armed with four teeth. antennae 9-segmented; the club distinct formed of the apical three joints. Pro-mesonotal shield about as broad as long with the anterior angles prominent and acute, the sides posteriorly with a small incision and beyond that produced backwards into a long, somewhat laminate spine on each side overhanging the metanotum, 1st node of pedicel smooth, viewed from side triangular, 2nd node globose, coarsely sculptured.

*Distribution:* Rajasthan—Dungarpur, Pokran (Chhotani and Ray)

*Range:* Throughout India. Myanmar and Tenasserin and extending to the Malayan sub-region.

*Remarks:* At once recognised by its hairy appearance and pro-mesonotal shield undivided, mesonotum produced backwards into long spines on each side overhanging the metanotum.

## 2. Tribe Tetramoriini Emery

*Tetramorium walshi* Forel

*Tetramorium walshi*, Forel *Ann. Soc. Ent. Belg.*, 34 (1890), 107, ♀ ♂.

*Material:*

6 exs, N.s.R., 20.8.84

Ants collected under stone.

*Diagnostic characters:*

Length ♀ - 2.5 mm.

Dull brown, abdomen black. Pilosity very dense and woolly, whitish in colour and somewhat concealing the sculpture. Head narrowed anteriorly. Posterior margin of clypeus produced between the basis of antennae. Mandibles broad with 5–7 teeth. Antennae 12-jointed, short, the scape and not reaching the top of the head. Thorax short and broad, curved and convex above, pronotum unarmed, the metanotal spine sub-triangular and acute at apex pointing obliquely back. Erect hairs on body trifold. First node of pedicel distinctly transverse, much broader than long. Petiole of 1st node nearly as long as node.

*Distribution:* Rajasthan—Dungarpur.

*Range:* India : Western India, Himachal Pradesh, Karnataka, Bengal and N.W. India.

Sri-Lanka (Chapman and Capco, 1951)

*Remarks:* Occurs in Western India but recorded for the first time from Rajasthan.

Recognised at once by its peculiar woolly appearance nearly all the hairs being trifold above the base.

### Zoogeographical Analysis of Ant fauna of Dungarpur

Universal	Indian sub-continent	Endemic
1. <i>Tapinoma</i> ( <i>Micromyrmex</i> ) <i>melanocephalum</i> (Fabr.)	1. <i>Leptogenys</i> ( <i>Lobopelta</i> ) <i>processionalis</i> (Jerdon)	1. <i>Aenictus</i> ( <i>Aenictus</i> ) <i>brevicornis</i> (Mayr)
2. <i>Camponotus</i> ( <i>Taenomyrmex</i> ) <i>compressus</i> (Fabr.)	2. <i>Tetramorium walshi</i> Forel	2. <i>Plagiolepis jerdoni</i> Forel
3. <i>Camponotus</i> ( <i>Orth-</i> <i>onotomyrmex</i> ) <i>sericeus</i> (Fabr.)		
4. <i>Tetraponera</i> ( <i>Tetraponera</i> ) <i>rufonigra</i> (Jerdon)		
5. <i>Meranoplus bicolor</i> (Guerin)		

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## Interaction of Earhead Bug, *Leptocorisa acuta* Thunb. and Certain Pathogenic Fungi on Deterioration in Rice Grain Quality

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**ABSTRACT:** Under natural conditions of *Leptocorisa acuta* infestation in the farmer paddy fields as well as at CRRRI farm six pathogenic fungi viz., sheath-rot fungus, *Acrocyndrium* (*Sarocladium*) *oryzae* Swada; brown spot fungus, *Helmithosporium oryzae* Breda-de-Haan; stack-burn fungus, *Alternaria padwickii* (Ganguly) Ellis; Bakane disease or foot-rot fungus, *Fusarium moniliforme* Synder & Hansen; *Fusarium graminearum* Schwabe and *Curvularia lunata* (Wakker) Boedigin were reported to be associated with the discolouration of the bug infested grains. Under artificial inoculation of the three test fungi, *A. oryzae*; *F. graminearum* and *C. lunata* on the developing grains of the potted rice variety Ratna along with the infestation of rice bug revealed that grain discolouration due to all the test fungi was enhanced significantly as compared to inoculation of any of the test fungi alone on rice variety Ratna. Thus rice bug, *L. acuta* was found to enhance the rice grain discolouration of the test fungi. © 2000 Association for Advancement of Entomology

**KEYWORDS:** Sheath-rot fungus, *Acrocyndrium* (*Sarocladium*) *oryzae*, brown spot fungus, *Helmithosporium oryzae*, stack-burn fungus, *Alternaria padwickii*, Bakane disease, foot-rot fungus, *Fusarium moniliforme*, *Fusarium graminearum*, *Curvularia lunata*, grain discolouration, rice earhead bug, *Leptocorisa acuta*.

### INTRODUCTION

Several bio-agents viz., bugs, thrips, stem borers, mites, white-tip nematode, pathogenic fungi and bacteria are known to deteriorate grain quality (Goto *et al.*, 1987; Prakash *et al.*, 1994; Rao and Prakash, 1995). Rice bugs have been recognised as one of the potential bio-agents to considerably deteriorate the quality of rice grains in the farmers paddy fields (Prakash *et al.*, 1995). These bugs, when feed on the developing grains during flowering cause injury to the paddy, as a result injured grains remain unfilled (chaff/sterile) or partially filled (ill-filled), whereas their feeding during milky grain stage cause **pecky rice** i.e. discolouration of the grains due to secondary infections of pathogenic fungi and bacteria (Prakash *et al.*, 1995; Rao and Prakash, 1995).

\*Corresponding author

Deterioration of rice grain quality is manifested in terms of ill-filled, deformed, bug-sucked and subsequent discoloured grains due to secondary infections of the pathogens (Rao, 1988; Rao *et al.*, 1994). Lee *et al.* (1986); Hallay *et al.* (1987) and Lakshmanan *et al.* (1992) have earlier studied the role of rice bugs and pathogenic fungi to cause grain discolouration in the paddy fields under different eco-climatic conditions. However, no adequate and systematic information is available on the interactions of the rice bugs and the pathogenic fungi deteriorating rice grain quality in India. Therefore, we studied the interactions of rice earhead bug, *Leptocorisa acuta* Thunb. with certain pathogenic fungi under field and net house conditions.

### MATERIAL AND METHODS

Two experiments were conducted to establish the interactions of rice earhead bug, *L. acuta* and certain pathogenic fungi known to cause grain discolouration.

#### Experiment 1

In this experiment association of pathogenic fungi with the bug infested & discoloured paddy grains was studied. Paddy grains of three varieties viz., Kalinga III (rainfed upland variety crossed between AC 540 × Ratna having 80 days duration), Ratna (irrigated medium land variety crossed between TKM 6 × IR 8 having 120 days duration) and Savitri (rainfed lowland variety crossed between Pankaj × Jagannath having 155 days duration) infested by *L. acuta* and discoloured due to subsequent infections of the pathogenic fungi were collected from the farmer paddy fields, and also from CRRI Farm during six wet seasons i.e. 1992, 93, 94, 95, 96 and 97. After each wet season randomly selected 1000 grains of each test variety were surface sterilised using 1% sodium hypochlorite and sterile water and plated on 2% Oat Meal Agar (OMA) Medium in the paired petri-plates @ 25 grains/petri-plate (8 cms dia.) for a period of 7 days at room temperature (23–32 °C) under aseptic laboratory conditions. Observations were made on the fungal growth on the plated grains after 7 days of plating. The fungi were isolated and identified. Per cent fungal infection of each isolated fungi was calculated using formula given below and presented in Table 1.

$$\text{Per cent fungal infection} = \frac{\text{Number of infected grains} \times 100}{\text{Number of plated grains}}$$

#### Experiment 2

In this experiment interactions of *L. acuta* infestation on the infections of three pathogenic fungi viz., sheath rot fungus, *Acrocyndrium (Sarocladium) oryzae* Swada; *Fusarium graminearum* Schwabe and *Curvularia lunata* (Wakker) Boedigin and subsequent discolouration of paddy grains were studied in the net-house conditions. Plants of rice variety Ratna were grown in the sterilized soil in the earthen pots to avoid grain discolouration due to mites and soil borne microbes and covered with double layered nylon cage designed for rearing the rice bugs (Rao and Prakash, 1995).

TABLE 1. Per cent infection\* of fungi developed on the plated bug infested paddy grains

Fungus	Kalinga III	Ratna	Savitri
<i>Acrocyldrium oryzae</i>	22.20 (14.23)	22.98 (15.26)	26.99 (20.63)
<i>Fusarium graminearum</i>	18.48 (10.03)	21.19 (13.06)	29.50 (24.24)
<i>Curvularia lunata</i>	10.72 (3.48)	18.19 (9.74)	20.22 (11.94)
<i>Helminthosporium oryzae</i>	12.28 (4.53)	0.84 (5.26)	0.34 (3.34)
<i>Alternaria padwickii</i>	6.96 (1.46)	8.21 (2.04)	12.85 (5.58)
<i>Fusarium moniliforme</i>	6.39 (1.23)	12.85 (4.96)	9.88 (2.96)
SEM $\pm$	0.69	0.90	1.13
LSD (0.05)	2.13	2.64	3.48

\* = Mean of six wet seasons (1992, 93, 94, 95, 96 & 97) data.

Data in parentheses are original values of Arcsin  $\sqrt{\text{percentage}}$  without parenthesis

The spore suspension of each of the test fungi containing  $10^6$  spores/ml was sprayed on the through leaf exile during late tillering stage of the plant. Newly developed adults of *L. acuta* were released in each cage a week after panicle emergence @ a pair of bug/panicle. There were ten cages for each test fungi coupled with bug infestation and ten cages for each fungi were kept without release of the bugs. Further, ten more cages were kept with only bugs release without any fungal inoculum. In addition, ten cages were also kept as control without any fungal inoculum and bug infestation. After grain maturation, panicles were collected and bug infested and discoloured grains were collected and counted. Per cent grain discolouration was calculated using formula as mentioned below and presented in Table 2.

$$\text{Per cent grain discolouration} = \frac{\text{Number of discoloured grains} \times 100}{\text{Number of healthy grains}}$$

## RESULTS AND DISCUSSION

Results presented in Table 1 revealed that six pathogenic fungi were known to cause grain dis-colouration viz., sheath-rot fungus, *Acrocyldrium (Sarocladium) oryzae* Swada; brown spot fungus, *Helmithosporium oryzae* Breda-de-Haan; stack- burn fungus, *Alternaria padwickii* (Ganguly) Ellis; Bakane disease or foot-rot fungus, *Fusarium moniliforme* Synder & Hansen; *Fusarium graminearum* Schwabe and

TABLE 2. Per cent paddy grain discolouration\* due to infections of pathogenic fungi and rice bug, *L. acuta*

Treatment	Kalinga III	Ratna	Savitri
<i>L. acuta</i> + <i>A. oryzae</i>	32.98 (29.67)	34.65 (32.33)	39.03 (39.67)
<i>L. acuta</i> + <i>F. graminearum</i>	30.68 (26.04)	33.74 (30.84)	35.36 (33.44)
<i>L. acuta</i> + <i>C. lunata</i>	24.28 (16.93)	30.42 (25.64)	31.22 (26.89)
<i>A. oryzae</i>	14.50 (6.29)	18.76 (10.38)	25.64 (18.67)
<i>F. graminearum</i>	17.12 (8.68)	20.89 (12.71)	28.81 (23.24)
<i>C. lunata</i>	19.05 (10.67)	25.27 (18.22)	28.18 (22.30)
<i>L. acuta</i>	6.21 (1.22)	7.76 (1.87)	7.95 (1.93)
Check (No infection/infestation)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEM $\pm$	1.24	1.77	1.96
LSD (0.05)	3.83	5.26	5.97

\* = Mean of three seasons (1995, 96 & 97) data.

Data in parentheses are original values of Arcsin  $\sqrt{\text{percentage}}$  without parenthesis.

*Curvularia lunata* (Wakker) Boedigin and were associated with the subsequent grain discolouration after infestation of rice earhead bug, *L. acuta*.

Per cent grain infections of all the isolated fungi was highest in rainfed lowland variety Savitri followed by Ratna and lowest in Kalinga III except in case of *H. oryzae* infections, which was highest in Kalinga III and lowest in Savitri. When compared among the fungi infections in Kalinga III, per cent fungal infection was highest by *A. oryzae* followed by *F. graminearum*, *H. oryzae*, *C. lunata*, *A. padwickii* and *F. moniliforme* and differed significantly except between *A. padwickii* and *F. moniliforme* (Table 1). Per cent fungal infections in Ratna and Savitri grains were also highest by *A. oryzae* and *F. graminearum* as observed in case of Kalinga III but thereafter, order of fungal infections differed with Kalinga III as *C. lunata* followed by *F. moniliforme*, *A. padwickii* and *H. oryzae* in Ratna and *C. lunata* followed by *A. padwickii*, *F. moniliforme* and *H. oryzae* in Savitri. Earlier, Douglas and Tullis (1950); Lee *et al.* (1986) and Hallay *et al.* (1987) reported that micro-organisms such as *Bipolaris oryzae*, *Curvularia lunata*, *Cercospora oryzae*, *Trichoconis caudata*, *Fusarium oxysporium*, *Alternaria alternaria*, *A. padwickii* and *Nematospora coryli* were associated with the feeding activity of the pentatomid bug, *Oebalus pugnax* and

subsequent rice grain discolouration in USA. However, in India sheath-rot fungus, *Acrocyldrium oryze* was found to be associated with the infestation of the rice earhead bugs, *Leptocorisa acuta* and *L. oratorius* (Lakshmanan *et al.*, 1992; Rao and Prakash, 1995).

Similarly, mean per cent grain discolouration of the paddy grains due to collective infections/infestations of the rice bug and three test fungi, *A. oryzae*; *F. graminearum* and *C. lunata* under artificial infestation/inoculation revealed that grain discolouration due to all the test fungi was enhanced significantly as compared to inoculation of any of the test fungi (Table 2). Further, grain discolouration was also recorded to be highest in long duration variety Savitri followed by Ratna, a medium duration variety and lowest in short duration variety Kalinga III in all the test combinations as well in treatments of fungus alone. This may be due to coinciding the exposure of the infected grains to more favourable climatic conditions for fungal development in this long duration variety.

Thus results of this experiment showed that infestation of rice earhead bug enhanced the grain discolouration caused by the test fungi. Also, highest grain discolouration was caused by *A. oryzae* followed by *F. graminearum* and *C. lunata* as found under natural field conditions. Earlier grain discolouration in rice was also reported to be enhanced by the interactions of the white-tip nematode, *Aphelenchoides besseyi* Christie with the seed borne fungi like *Sarocladium oryzae* (Rao and Kauraw, 1990a), with *Fusarium moniliforme* (Rao and Kauraw, 1990b) and also with *Curvularia lunata* (Rao *et al.*, 1994). Rao and Prakash (1996) also found rice tarsonemid mite, *Steneotarsonemus spinki*. Smiley to enhance rice grain discolouration along with the infections of fungi like *A. oryzae* and *F. graminearum*.

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## On the Description of Female of *Acromantis montana* Giglio-Tos from Kumta, Karnataka, Western Ghats (Mantodea : Hymenopodidae)

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**ABSTRACT:** Female of the mantis *Acromantis montana* is redescribed from a specimen collected from Kumta, Karnataka. It also forms first report of this species from Western India. © 2000 Association for Advancement of Entomology

**KEYWORDS:** Mantodea, Hymenopodidae, *Acromantis montana*.

Genus *Acromantis* under family Hymenopodidae includes 4 species in India. Out of these only *A. insularis* is known from Western Ghats. The other three species are distributed as follows: *A. nicobarica* is known from Great Nicobar Island, *A. oligoneura* is known from Assam and Meghalaya while *A. montana* is known from Arunachal Pradesh, Meghalaya and Tripura. (Mukherjee *et al.*, 1995).

Recently a specimen of *A. montana* Giglio-Tos was collected from forested area of Santegulli, Kumta, Karnataka (collector Nilesh Rane, date 14 October 1998). It is a female individual and has an interesting set of characters that need to be described, especially because the female of this species has not been adequately described earlier. This will also extend the range of *A. montana* to Western India as all the previous records are from North-East India. Outside India the species is known from Java, Indonesia.

**Description:** Head : Tubercle above median ocellus absent (very small spiniform tubercle generally present in male), upper angle of frontal sclerite pointed (spiniform), ocelli large, vertex black.

**Thorax:** Pronotum dorsally brownish, excepting black at anterior most part of prozona, lateral borders brown, with black tubercular spines (4 in prozona, 7–8 in metazona). Ventrally, prozona more blackish to black, but metazona less blackish (metazona of prosternum black in male). Parts of fore legs blackish with scattered black dots; coxa with 6–7 brownish, minute tubercular spines; femur with 4 discoidal, 12 internal (6 long, 6 short) and 4 external spines; discoidal and longer internal spines

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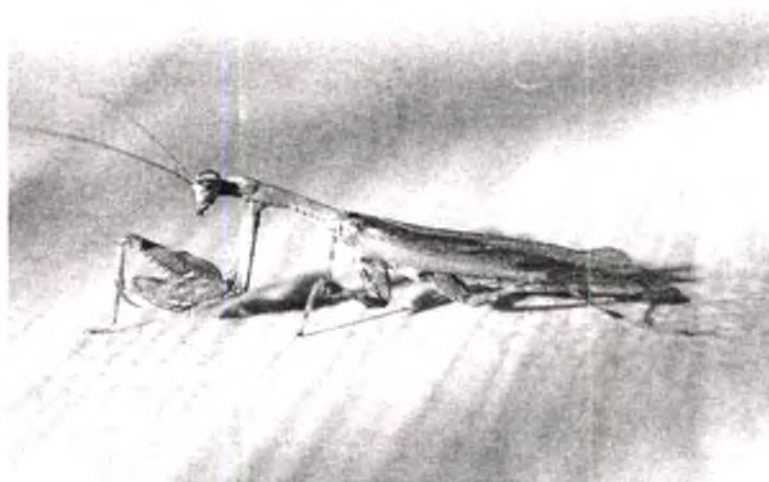


FIGURE 1. Photograph of live female mantis, *Acromantis montana*.

are entirely black; tibia with 13 internal spines, all black at tips only. In fore-wing, costal area opaque, brownish green (green in life), densely reticulate; 4 longitudinal oblique veins with brown, lengthwise patch at distal length (no such patch on oblique veins in male). In hind-wing, the costal area and distalmost apex brown. Middle and hind legs triannulated black; middle and hind femora with ventral lobes (1 proximal, 1 distal), these lobes are larger in middle femora (see Fig. 1)

*Measurements:* Total length 24 mm, prozona 2.5 mm, metazona 5.0 mm, anterior coxa 5.0 mm, femur 6.5 mm, tibia 3.0 mm, fore-wing 20.0 mm, hind-wing 17.0 mm. The specimen is deposited at the Department of Zoology, Modern College, Pune 411005.

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## ***Armigeres joloensis* (Diptera : Culicidae), a Rare Mosquito in Upper Assam: First Report from India**

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**ABSTRACT:** *Armigeres* (*Armigeres*) *joloensis* a rare mosquito known so far only from Philippine island, was recorded for the first time in India from district Dibrugarh of upper Assam. © 2000 Association for Advancement of Entomology

**KEYWORDS:** Mosquito, *Armigeres*, New Country record.

Mosquito genus *Armigeres* Theobald, primarily of Oriental region, is also common in the Australasian region. Mosquitoes of this genus occur chiefly in forested localities, breed in microhabitats e.g. tree holes, bamboo stumps etc. and are usually day biters particularly in crepuscular period. A few species of *Armigeres* can transmit *Setaria digitata*, the cattle filaria worm (Verma *et al.*, 1971) in addition to bancroftian filariasis and encephalitis virus (Manson-Bahr and Apted, 1989). Of the total 46 species of *Armigeres* mosquitoes recorded worldwide, 14 have been documented in India (Knight and Stone, 1977).

The genus *Armigeres* is divided into two subgenera viz., *Armigeres* and *Leicesteria*. Barraud (1934) documented 4 species belonging to subgenus *Armigeres* from India viz., *kuchingensis*, *theobaldi*, *aureolineatus* and *subalbatus* (= *obturbans*). Four varieties of *Armigeres kuchingensis* viz., *dibrugarhensis*, *shillongensis*, *durhami* and *nongpohensis* were also described from India and all the four were recorded from the north-eastern region (Barraud, 1934). Later, Thurman (1959) gave species status to *Armigeres durhami* and synonymised *dibrugarhensis* and *shillongensis* with it and *nongpohensis* with *Armigeres kuchingensis*.

The north-eastern region of India, considered as one of the 18 'hot spots' for biodiversity in the world (Khoshoo, 1994) is rich in mosquito fauna also. Our Centre is carrying out regular mosquito fauna surveys in different north-eastern states. During, a recent survey conducted on 19th September 1998, few mosquito larvae were collected from a cut tree hole in the Saraipung forest range of Dibrugarh district, Assam. This

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FIGURE 1. Male genitalia of *Armigeres joloensis* (Original magnification 100×).

evergreen tropical forest range is situated at the border of Dibrugarh district, Assam and Tirap district of Arunachal Pradesh at an attitude of 158 meters from msl. Larvae were reared in the laboratory upto adult stage and identified as mosquitoes of genus *Armigeres* and subgenus *Armigeres*. Genitalia of the male specimens were dissected after treating with 10% KOH and slide mounted using Hoyer's solution for species identification.

The male genitalia of the specimens were found completely different to the previously described species of subgenus *Armigeres* from India (Barraud, 1934). However its spatulate, striated claspette setae and phallosome (Fig. 1) closely resembled with that of *Armigeres (Armigeres) joloensis* (Ludlow) reported only from Philippines (Ludlow, 1904; Bohart, 1945). Ludlow (1904) first described the species, *Armigeres joloensis* as *Desvoidea fusca* var. *joloensis* from Philippines (Type loc: Jolo Jolo island, (Sulu island), Philippines). Subsequently, *Armigeres joloensis* was listed as a synonym of *Armigeres subalbatus* (= *obturbans*) (Bohart, 1945). Later, Stone and Thurman (1958) resurrected *Armigeres joloensis* from synonymy with *Armigeres obturbans* and gave it separate species status. Only one type specimen (male) of *Armigeres joloensis* was deposited with United States National Museum (USNM), Washington DC. Its female, larval and pupal stages still remained undescribed.

We have recorded this rare species of mosquito for the first time in India. Two male genitalia slides were deposited in the museum of CRME (Centre for Research in Medical Entomology) Madurai and one specimen with its genitalia mounted on slide was kept at RMRC (Regional Medical Research Centre) Dibrugarh museum. Efforts

are being made to collect more specimens of *Armigeres joloensis* to study its medical importance and role in food chain.

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## **Antifeedant and Growth Inhibitory Effects of Neem seed Kernel Extract on *Ailanthus* defoliator, *Eligma narcissus indica* Roth. (Lepidoptera : Noctuidae)**

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**ABSTRACT:** Methanolic extract of neem seed kernel (NSKE) was evaluated for its antifeedant and growth inhibitory effects against last instar larvae of *Ailanthus* defoliator, *Eligma narcissus indica*. The results indicate feeding deterrence and growth inhibition. Food consumption, weight gain and relative growth rate (RGR) were decreased significantly with increasing extract concentrations used. The co-efficient of approximate digestibility (CAD) and efficiency of conversion of ingested and digested food (ECI & ECD) showed increasing trend. Larval feeding on NSKE treated food resulted in various degrees of growth disruption in pupal and adult morphogenesis in a dose-dependent manner. © 2000 Association for Advancement of Entomology

**KEYWORDS:** *Ailanthus* defoliator, *Eligma narcissus indica*, Roth., last instar larva, Neem seed kernel extract (NSKE), feeding deterrence, growth inhibition.

The potential of several secondary chemical compounds in plants to disrupt specific physiological mechanisms involved in nutrition, reproduction, metamorphosis and behaviour of insects could be exploited in the control of insect pests as an eco-friendly alternative to the conventional pesticide use. In this context, use of extracts of such plants like Neem (*Azadirachta indica* A. Juss.) is now gaining momentum since several active insecticidal neem compounds, including azadirachtin, are feeding inhibitors and growth disruptors for most insect orders (Schmutterer, 1990). *Ailanthus triphyssa* is a fast growing soft wood tree of considerable economic importance in Kerala for match, packing case and paper pulp industries. The major monophagous pest of *Ailanthus* in Kerala, as elsewhere in India, is *Eligma narcissus indica* Roth (Varma, 1986). This paper reports the effects of neem extract on nutrition and growth in this serious defoliator insect in forest plantations, homesteads and nurseries of *A. triphyssa* in Kerala.

A culture of *E. narcissus indica* was maintained as described earlier (Joseph, 1999). Final (sixth) instar larvae, 3–6 h after moulting, were used in the presence experiments. NSKE was prepared according to Joseph and Karnavar (1993). Different concentrations (250, 500, 750 & 100 ppm) were prepared by dissolving required quantities (25, 50, 75 & 100 mg) of NSKE in 2 ml of methanol separately and making up each 100 ml using distilled water. Fresh *A. triphyssa* leaves collected from the field



FIGURES. 1-2 & 5-11. Selected examples of developmental abnormalities in *E. narcissus indica* due to neem seed extract feeding by VI instar larvae (1: larva showing early symptoms of body shrinkage; 2: Larva showing pronounced body shrinkage; 5, 6 & 7: Larval-pupal intermediates with larval cuticle patches, head capsule and thoracic legs in the anterior region; 8 & 9: Abnormal pupae with malformed cuticle and appendages; 11: Malformed small sized adult with reduced crumpled wings and deformed antennae).

FIGURES. 3, 4, 10 & 12 Normal insects from control. (3: Larva 4: Pre-pupa. 10: Pupa. 12: Adult.)

were dipped in different concentrations of NSKE separately, left to dry in air, weighed and then placed in separate wire-mesh cages (25 × 45 cm) along with weighed larvae (5 larvae/cage; 5 replicates/each concentration) for 24 h. The uneaten leaf parts thereof were removed and weighed. The larvae were allowed to remain in their cages without leaves for about 2 h so that they could void all their faeces. The larvae and faeces were then separated and weighed. The experiment was continued for one more day with neem treated fresh leaves and on the subsequent days the larvae were fed on untreated leaves till the termination of the feeding phase. The control larvae were fed on methanol: water (1 : 49 by volume) solvent treated fresh leaves for the first two days and then on untreated leaves. A correction factor for leaf desiccation was calculated each time by placing leaves under similar conditions without larvae. Both experimental and control larvae were observed on their development to pupae and adults. The food consumption and utilization efficiencies were calculated as described by Gordon (1968), but based on fresh weights (Fagoonee, 1984) as it was difficult to obtain dry weights for larvae on successive days. Metrical data were subjected to one-way classification of analysis of variance followed by Kramer's multiple range test (KMRT).

TABLE 1. Effect of NSKE (foliar application) on consumption index (CI), relative consumption rate (RCR), co-efficient of approximate digestibility (CAD), efficiency of conversion of ingested and digested food (ECI & ECD), weight gain (WG) and relative growth rate (RGR) in the final instar larvae of *E. narciissus indica*

Concentration (ppm)	CI	RCR	CAD	ECI	ECD	WG (mg/larva)	RGR
1000	1.58 ± 0.24*	0.17 ± 0.001*	0.61 ± 0.044*	0.64 ± 0.091*	1.06 ± 0.174*	78.19 ± 12.98*	0.57 ± 0.124*
750	2.03 ± 0.10*	0.36 ± 0.0004*	0.50 ± 0.00*	0.49 ± 0.023*	0.99 ± 0.044*	138.20 ± 4.93*	1.00 ± 0.070*
500	2.40 ± 0.12*	0.59 ± 0.015*	0.50 ± 0.001*	0.42 ± 0.021*	0.84 ± 0.041†	215.21 ± 8.89*	1.58 ± 0.129*
250	3.60 ± 0.12**	1.09 ± 0.041*	0.51 ± 0.001*	0.28 ± 0.0001*	0.54 ± 0.025*	296.55 ± 7.96*	2.14 ± 0.121*
Control	3.80 ± 0.10	1.44 ± 0.056	0.33 ± 0.001	0.26 ± 0.001	0.79 ± 0.040	417.76 ± 8.12	3.04 ± 0.168

Values represent mean ± SD of 5 replications \*  $P < 0.01$ ; \*\*  $P < 0.05$ ; † Not significant

TABLE 2. Effect of NSKE (foliar application) on larval survival, growth and development in *E. narcissus indica*

Concentration (ppm)	Surviving larvae on day 5	Number of resultant insects				Larval period (days)	Pupal Period (days)
		Normal Pupae	Larval-pupal intermediates	Normal moths	Deformed moths		
1000 <i>n</i> = 25	9(36)	2(8)	4(16)	0(0)	2(8)	8.3 ± 0.68	14.5 ± 0.71
750 <i>n</i> = 25	11(44)	3(12)	7(28)	1(4)	2(8)	8.0 ± 0.75	14.2 ± 1.26
500 <i>n</i> = 25	16(64)	8(32)	6(24)	2(8)	5(20)	7.5 ± 0.65	13.8 ± 0.70
250 <i>n</i> = 25	23(92)	19(76)	3(12)	12(48)	6(24)	7.0 ± 0.30	12.9 ± 0.70
Control	24(96)	23(92)	0(0)	22(88)	0(0)	6.6 ± 0.37	12.3 ± 0.75

*n* = total number of sixth instar larvae used. Figures in parentheses indicate the percentage.

Larval feeding of NSKE-treated foliage resulted in persistent feeding deterrence (Table 1) and growth inhibition at different levels during metamorphosis (Table 2 & Fig. 1–2 & 5–11) in *E. narcissus indica*. These results are consistent with the earlier reports on various lepidopterous species (Fagoonee, 1984; Arnason *et al.*, 1985; Barnby and Klocke, 1987; Sridhar and Chetty, 1989; Koul and Isman, 1991; Jhansi and Singh, 1993; Sahayaraj, 1998). Feeding deterrence of neem compounds is explained by behavioural antifeedant effect due to perception by the peripheral chemoreceptors in insects (Schoonhoven, 1981; Simmonds and Blaney, 1984). Feeding deterrence is also shown to be due to the action of neem compounds on the centres that control gut mobility and metabolism (Schluter and Schulz, 1984; Mordue *et al.*, 1985; Dorn and Trumm, 1993). The functional property of fatty acids in the NSKE plays an inhibitory role too on feeding (Sridhar and Chetty, 1989). Significantly higher ECI and ECD values as that of control were obtained at all concentrations in the present study as reported in *Crocidolomia binotalis* (Fagoonee, 1984) and *Spodoptera litura* (Sahayaraj, 1998). Neem related high ECI and ECD values reflect compensations for feeding inhibition (Fagoonee, 1984). NSKE, at all concentrations in the food, tends to increase CAD from that of control insects in the present study (Table 1). This is in conformity with the earlier reports (Fagoonee, 1984; Arnason *et al.*, 1985; Barnby and Klocke, 1987). High CAD is attributed to the greater retention of food in the midgut due to inhibited gut mobility (Mordue *et al.*, 1985; Dorn and Trumm, 1993).

The present findings show that NSKE is effective in inhibiting larval growth and inducing pupal and adult deformities in *E. narcissus indica* (Table 2 & Fig. 1–12). Such growth inhibitory effects of neem, ranging from delay in moulting with the production of deformed insects, to the complete inhibition of growth at higher doses, have been reported in various insect species (Schmutterer, 1990). Dose-dependent inhibition of larval growth and morphogenesis in *E. narcissus indica* could be accounted due to reduced physiological age brought about by reduced food intake and weight gain as reported in *Heliothis virescens* (Barnby and Klocke, 1987).

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## **Indo-Australian Ormyridae (Hymenoptera : Chalcidoidea)**

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